ABSTRACT OF THESIS

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Title of Thesis "ANALYSIS OF HETEROSIS IN MOUSE CROSSES"

Heterosis was found on crossing inbred strains of mice, in litter size, birth weight and weaning weight. No example of a maternal-offspring incompatibility was found in any of these characters.

Litter size, on crossing, was largely under maternal determination. The occurrence of heterosis depended on the strains used as female parents, some strains showing an increase on crossing. The increase was never very large in this experiment. Litter size at implantation was completely maternally determined on crossing. Heterosis in litter size, when it occurred, was due to reduction in early post-implantation mortality, (except in one case, where reduction in peri-natal mortality was invoked). The reduction in mortality was interpreted as evidence for inbreeding depression in early post-implantation survival of the inbred strains.

Heterosis in foetal weight at birth and at 17 days' gestation was found to occur to a very marked degree. Placental weight at 17 days' gestation showed heterosis on crossing. Various maternal effects on foetal weight and placental weight were isolated statistically. Placental size was not implicated to a large degree in the mediation of maternal effects on foetal growth. It is suggested that differences between reciprocal crosses in foetal weight may be explained on haemodynamic principles, on consideration of the possible basis of observed maternal effects.

Heterosis in foetal weight and in weaning weight indicated that crossbreds have an inherently faster rate of growth than inbreds in the same maternal environment. The nutritional implications are discussed.

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ANALYSIS OF HETEROSIS IN MOUSE CROSSES

Thesis submitted to the Faculty of Science
of the University of Edinburgh for the
Degree of Doctor of Philosophy

by

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CONTENTS

CHAPTER I

INTRODUCTION

The rediscovery of Mendel's work early in this century stimulated genetical interpretation of inbreeding depression and its complementary phenomenon, heterosis. Although plant and animal improvement on an unprecedented scale resulted from the exploitation of heterosis in breeding practice, no comprehensive hypothesis is yet available which will satisfactorily explain the various and sometimes conflicting observations made in crossbreeding experiments. The theory of the inheritance of quantitative characters by many genes and the modification of its premises to secure agreement with observation is the subject of many recent reviews (Bowman, 1959; Schnell, 1961, etc.). Another such review being considered superfluous, only an outline of the main hypotheses will be given here.

Two principal hypotheses were proposed to explain the genetic basis of heterosis, these being reasserted with some modification over the years. One is the dominance theory according to which dominant genes cooperate to produce heterosis by virtue of the fact that deleterious recessive alleles are masked by their superior alleles in hybrids which possess a greater array of dominant alleles than either parent and thus show heterosis. The theory is based on the observed correlation between dominance and favourable effects. The other theory invokes hybridity per se as the

cause of heterosis and assumes overdominance to be effective at least at some of the loci involved in heterosis. Far from being mutually exclusive the two theories differ essentially only in the degree of dominance concerned. The difference between the two theories may be vanishingly small in their practical consequences when linkage is taken into consideration. Simple dominance with repulsion linkage is indistinguishable from overdominance in its effect on crosses. The two may however be partially distinguished in later generations. Robinson et al. (1960) have outlined a test which is based on the change in magnitude of the additive genetic variance and the dominance variance from the F_2 to later generations. Their results tended to support the hypothesis that linkage did bias estimates of dominance variance in earlier generations.

More complex genetic interactions than those visualised in the former theories have been exposed in studies on the effects of inserting chromosome rearrangements on Drosophila pseudoobscura in different genetic backgrounds (Dobzhansky, 1954) and in analysis of effects of chromosome substitution in D. melanogaster (Robertson & Reeve, 1953). Non-allelic interactions have also been reported by Jinks (1955) in analysis of diallel plant crosses. Are such interactions of importance in the causation of heterosis? Within populations the amount of epistatic variation is likely to be small usually and Falconer (1960a) considers that it is seldom necessary to distinguish it from other sources of non-additive genetic variance with reference to a single population. However when crosses between differentiated populations are considered non-allelic interactions may assume importance. Theory of the evolutionary derivation of heterosis invokes epistatic interaction to a major degree and introduces

the idea of "loss of fitness" in crosses between populations. As my study was originally designed to explore the possibility of the occurrence of incompatibilities between strains of mice on crossing, a situation which could result in "negative heterosis", it is important to appreciate the basis and implications of current theory.

The idea that interpopulation hybrids may be less fit than the parent populations in wide crosses has been proposed by Mather (1943, 1955) on the basis of his "balance" theory. That gene arrays in naturally outbreeding species are mutually adjusted to minimise the possible disadvantageous effects of segregation on fitness is referred to as "balance". Mather visualises a polygenic system in which many different balances of genic combinations are possible. These combinations are adjusted to mediate phenotypes adjusted to the requirements of the population and will have been adjusted optimally by natural selection. In outbreeding species, where naturally occurring genotypes will be nearly always heterozygous, natural selection will favour those genic combinations which combine in homologous pairs to give good "balance". This is called "relational balance". The theoretical consequence of crossing two strains of an outbreeding organism is that the hybrid will contain two polygenic combinations not previously selected for good relational balance and thus may show lower fitness than either parents.

Similarly, on the basis of "coadaptation", a theory which visualises the genetic structure of a natural population evolving as a whole, interpopulation hybrids may not be expected to show heterosis in fitness (Dobzhansky, 1950) because the genotypes of the F_1 represent two differently coadapted gene complexes.

Critical evaluation of experimental evidence relevant to these theories will not be attempted in the light of the divergent interpretations of observed results. MacFarquhar and Robertson (1963) have comprehensively reviewed this aspect of the subject and have made a systematic analysis of crosses between populations of D. subobscura. No evidence for "coadaptation" was found and these authors indicate that systematic analysis of the whole question of effective differences between populations is necessary on the basis of crossing populations rather than analysis of the effects of chromosome substitution, which so far has furnished the most convincing evidence of coadaptation. It should be understood that the theories of balance and coadaptation have been developed in the context of evolution and that any implications of these theories are made on genetic premises. Thus the expectation of lack of heterosis on crossing differentiated populations is based on gene-gene interactions, that is, the gene complement of the hybrid as such is the determining factor.

A very remarkable instance of negative heterosis in mice was reported by Mason et al. (1960). They found on crossing four strains of mice that "all significant differences from parental means for characters concerning growth and body weight were negatively heterotic". This negative heterosis was attributed to "a conflict between the physiology of the mother and the genotype of her crossbred offspring". This the authors regarded as resulting from the fact that the four strains "displayed conflicting physiological patterns" which "were mostly additive in their inheritance". Although the authors did suggest the nature of one such maternal-offspring incompatibility, the possible genetic and physiological implications were far from clear. Bowman (1958) found a similar though

less marked case. These examples of apparent loss of fitness in crossbred mice were what prompted the present investigation of the reproductive performance of mice. If the phenomenon could be confirmed, the genetic and physiological mechanisms might be investigated and it was thought that the elucidation of these might be relevant to the ideas of "balance" and "coadaptation" in the evolutionary context.

Maternal-offspring incompatibilities on quantitative characters had not been previously reported and would have practical consequences in breeding programmes designed to maximise hybrid vigour. Bowman (1962), in an experiment for recurrent selection for litter size in mice, found that the litter size of hybrid females, which was the criterion of selection, was lower than that of either parent strain in three cycles of selection and did not reach the mid-parent value in the fourth and last cycle of selection.

A maternal-foetal incompatibility due to a "single-gene antagonism" between mother and foetus in the mouse has been described by Hollander and Gowen (1959). When mothers were homozygous for the recessive gene "hair-loss", heavy mortality occurred post-natally among phenotypically normal offspring, "hair-loss" young being unaffected. A syndrome including inferior growth and fragile bones was associated with mortality. The effect was a prenatal one as fostering normal young gestated in hair-loss mothers did not alleviate the syndrome. The nature of the antagonism is yet unknown. A nutritional explanation was suggested on the basis of certain observations made on morphological features of the syndrome (Hollander, 1960). Attempts to find evidence of an immunogenetic effect have not been successful (McLaren and McKenzie, unpublished). However a single gene model seemed

unlikely to fit the situations described by Mason, Bogart, et al. (1960) or by Bowman. (1958)

My study was primarily instituted to find maternal-offspring incompatibilities by systematically crossing inbred strains of mice, to define such interactions, if found, in physiological terms and to evaluate the results in terms of heterosis. Heterosis on crossing breeds or strains of mammals is operationally defined as the deviation of the mean of reciprocal crosses from the mid-parent value. This deviation can be negative if a maternal-offspring incompatibility occurs in one of the reciprocal crosses. As will be seen there was no evidence to indicate that any such incompatibility occurred and only one cross was further explored in this relation. The preliminary results of crossing strains indicated that the orthodox situation held i.e. heterosis was evident.

Attention was turned to the study of the heterosis found. To elucidate the means by which heterosis was achieved became the object of my study. The characters studied were all subject to maternal effects. The design of the analysis can be visualised by considering two strains, say A and B, which are crossed reciprocally. The first comparison will be between AB progeny and A progeny in the A maternal environment and the second between AB progeny and B progeny in the B maternal environment, for it is these differences which determine whether heterosis, as operationally defined, is attained. Analysis of these differences will of course allow a third comparison to be made i.e. of progeny AB in two different maternal environments. Reciprocal differences give the simplest evidence that maternal effects occur. The physiological basis of such maternal effects is far from satisfactorily explained. Little is known

as to how foetal growth is restricted in some maternal environments relative to others or how the size of the mother can act to increase or restrict embryonic growth. This third comparison is also relevant to a wider genetical problem i.e. the effect of differing environments on a constant genotype. Dickinson (1960) found that maternal effects applied very early in life could have a profound effect on later growth when the maternal effect no longer operated. The material available in this experiment proved extremely suitable for the analysis of the nature of maternal effects on quantitative characters.

CHAPTER II

DETAILS OF PRELIMINARY EXPERIMENT

Strains used

Six inbred strains of mice were systematically crossed. The strains used were the following: CBA/Fa, C57/Fa, A/Fa, RIII/Fa, JU/Fa, and "KL". The last two strains are of local origin ; JU is a listed strain (Standardized Nomenclature for Inbred Strains of Mice, 1960) and had been inbred by full-sib mating for 30 generations when this study began. KL is not a listed strain; it originated from a four-way cross of the first four strains mentioned above and had subsequently been through over 40 generations of full-sib mating. The first four strains have been maintained in this laboratory by full-sib mating since 1947 when they were originally obtained as inbred stocks.

Crossing scheme

The crossing of the six strains in all possible combinations in an orthogonal six-by-six diallel scheme gave data on 15 between-strain crosses and their reciprocals. Six types of within-strain matings were represented in the diagonal as controls. It was proposed that each of the 36 cells of the diallel be represented by between 30 to 40 single-pair matings. It was desirable to have all matings contemporaneous but this was impracticable, firstly because some strains were difficult to propagate and secondly because of the exigencies of space. The procedure was as follows. Inbred females were mated at eight weeks of age in single-pair matings. Data were collected on the first two litters of each mating and as far as

possible crosses and controls involving females of one particular strain were mated contemporaneously. Table 1 shows the number of litters recorded in a period of fifteen months.

Characters studied

The characters recorded were:

(i) the number of young in each litter at birth; the number alive will be referred to as "litter size".

(ii) the collective weight of live young in each litter at birth.

(iii) the number of live young in each litter on weaning at 21 days and the weight of each mouse at weaning.

These characters were chosen to compare a wide range of inbred and crossbred genotypes in inbred maternal environments and were thought adequate to detect the expression of any maternal-offspring incompatibility.

Presentation of results

The preliminary experiment described above yielded results which led to further studies of the physiological aspects of each character. Three characters will be treated separately in Chapters III, IV and V. The results of the preliminary experiment will be presented at the beginning of each Chapter for a single character and this will be followed in each case by full presentation of subsequent studies on that particular character.

CHAPTER III

LITTER SIZE

The effect of crossing inbred strains on litter size

Results

The mean litter sizes (with standard errors) of all the inbred and crossbred matings are given in Table 2. The effect of crossing on litter size is illustrated in Fig. 1. Here the litter sizes on crossing females of one particular strain to several strains as males are plotted relative to the litter size of the strain as common female parent. If litter size is completely maternally determined then no significant deviations from the inbred level are expected. Two features are obvious. Firstly the size of crossbred litters was not in any instance significantly lower than that of inbred litters in the same maternal environment i.e. no example of negative heterosis was found. Secondly, the litter sizes of the strains used as males bore no general relationship to the size of crossbred litters i.e. the genes contributed by the father on crossing did not influence the degree of heterosis exhibited. The extent to which the size of crossbred litters is under maternal control can be seen from the relatively narrow range of litter sizes about the value of the common maternal parent in each comparison. What contribution did the heterozygosity of litters make to litter size on crossing strains? The mean litter size of all crossbred litters having a common strain as female parent was compared to the litter size of that maternal strain. The results are shown in Table 3 for both parities. (Parities have been shown separately in Fig. 1 and Tables 1, 2 and 3 as the effect of parity on litter size differed

between strains. The litter size of JU females was influenced by the effect of concurrent suckling of first litters and is not included in Table 3. This effect is reported in the Appendix. Supplementary data on first parity are included instead, comprising two contemporaneous groups of JU females, one group of 50 mated to JU males and the other group of 50 mated to C57 males). No effect of crossbreeding in the litter was found in the litter-size of JU females. A very slight but insignificant effect was found in litters of CBA and KL females. The effect of crossbreeding in litters of C57, RIII and A females was to increase litter size over the inbred maternal level by about one mouse.

Discussion

Maternal effects of severe degree were evident in the above experiment, yet the extent to which litter size was restricted to the maternal level on crossing was dependent on the strain as female parent. Previous observations on crosses between inbred lines and strains of mice supports this. Fortsthoefel (1954) on outcrossing females of the BALB/c strain recorded a significant increase of over two mice in the mean litter size of crossbred litters over the litter size of the maternal strain. Butler (1958) observed a similar increase with the same strain on crossing, but found no significant increase on outcrossing C57 females. Eaton (1953) crossed nine inbred strains. The increase due to crossbreeding of litters was found to vary from strain to strain, estimates ranging from 0.3 to 2.5 extra mice over the means of maternal strains being recorded. On crossing lines inbred for six generations Eaton (1953) found little effect of crossbreeding in the F_1, a result which agrees with Roberts (1960) who found

only a slight effect of crossbreeding in the F_1 on crossing partially inbred material.

In a character subject to maternal effects heterosis in the F_1 is attributable to the non-maternal component of the character, the maternal component being at the inbred level. Maternal restriction of litter size in inbred mothers irrespective of the heterozygosity of the young is apparent but the degree to which litter size is restricted seems to depend on the genotype of the maternal strain. What factors are responsible for the suppression of the effect of heterozygosity in some maternal environments and its expression in others? The next experiment was designed to answer this question.

ANALYSIS OF THE COMPONENTS OF LITTER SIZE

Introduction

The number of live young at birth is determined by the following series of component characters:

(i) the number of eggs ovulated.

(ii) the proportion of eggs fertilised.

(iii) the number of eggs implanting.

(iv) the proportion of implants surviving to term.

(v) the proportion of peri-natal mortality.

If inbred females are crossed and show an increase in litter size over the inbred level, this increase will be equal to the sum of the deviations from the values of these component characters in within-strain matings. Previously published work provides some examples of such deviations. On outcrossing females of an inbred strain Krzanowska (1960) found a higher proportion of fertilised eggs than in within-strain matings. This type of effect would be detectable as reduction in pre-implantation mortality on crossing but this has not been observed in other experiments. Lyon (1959) found no increase in the proportion of eggs implanted on crossing three strains. Effects of crossing on post-implantation mortality have been observed. Lyon (1959) found that post-implantation mortality was reduced in between-strain matings.

This experiment was designed to determine at which stage or stages crossbreeding in the litter acted to increase litter size in between-strain matings and to explain the difference between strains in the response of litter size to crossing.

Method

Reciprocal crosses between CBA and C57 and between RIII and JU strains were compared to maternal parent strains. These strains were chosen for crossing mainly because they were productive enough to yield sufficient material for experimental work. The combination of RIII and JU strains in reciprocal crosses was made because these strains were so different in litter size and provided a contrast with the other combination of CBA and C57 strains which were equal in litter size. Inbred females were mated at eight weeks of age in harems of three females to one male. Females were dissected 17 days after insemination, indicated by the presence of a vaginal plug. Counts were made of (1) the number of corpora lutea, as a measure of the number of eggs ovulated, (2) the number of live embryos, and (3) the number of dead implants. Dead implants were classified on a morphological basis to indicate the stage of gestation at which death occurred. The difference between (1) and the total number of implants given by the sum of (2) and (3) indicated pre-implantation losses. Post-implantation losses were indicated by (3).

The classification of post-implantation losses was based on the appearance of the dead implants. Those represented by "moles" were scored as "early" deaths. This class comprised the majority of losses. Any implant which was represented at 17 days by an undersized placenta without a recognizably formed embryo was scored as a "middle" death. Dead embryos of recognizable form were scored as "late" deaths. The latter two classes were represented by few implants and will be referred to as "later" deaths.

Results

The results are presented in Table 4. Crossing did not influence

either the number of eggs shed or implanted by inbred females. There was no significant difference between the means of the number of corpora lutea or between the means of the number of implantations for two groups of females from each strain. Differences between strains for these two component characters were evident. Litter size at implantation was completely determined maternally.

Increases in the number of live young at 17 days' gestation on crossing were found. Crossbred litters of CBA females showed an increase of 0.5 ± 0.34, of C57 females showed an increase of 0.3 ± 0.31, of RIII females showed an increase of 0.6 ± 0.35 over inbred litters while crossbred litters of JU females showed a decrease of -0.1 ± 0.30. Only one increase, that in RIII females, approached statistical significance at the 5% level.

What is interesting is that in three strains the observed increases in litter size at birth on crossing these strains (presented above) were reflected by increases of similar magnitude in the number of live young at dissection. This can be appreciated by comparison of Table 3 (first parity) and Table 4, good agreement being obtained between the two experiments for CBA, RIII and JU strains. This agreement is not found for the C57 strain where inbred litters are smaller at birth. Do C57 inbred litters suffer higher perinatal mortality than crossbred litters? The data available from the first experiment are presented below.

Perinatal deaths in inbred and crossbred litters of C57 females

Litters	Total no. born	No. dead at birth	% perinatal loss
Inbred	259	8	3.1
All crossbred	1247	8	0.6

Dead embryos are found at birth more frequently in inbred litters but the proportion is much too small to explain the observed increase in the number of live young at birth on crossing C57 females. Mothers eat dead young after birth and this makes it difficult to obtain reliable data on perinatal losses.

Attention is now turned to the relative viability of inbred and crossbred embryos in the post-implantation stage. Increases in the number of live embryos at 17 days' gestation were observed on crossing inbred females but these were not statistically significant in terms of litter size. Significant reductions in mortality on crossing need not necessarily be reflected in statistically significant increases in litter size. This will depend on the proportion of mortality on the one hand and on the mean and variance of litter size on the other.

The total number of deaths in the post-implantation period is shown on the left-hand side of Table 5. The agreement of the two ratios of dead implants to total implants for inbred and crossbred litters of each strain was tested by a χ^2 test. χ^2 was significant in two comparisons. Testing the overall ratios took no account of the heterogeneity for degree of loss between litters within the inbred and crossbred groups. To get an estimate of this heterogeneity litters were combined in threes to give a mean expectation of about 2 for dead implants and groups were tested for heterogeneity. No significant between-litter heterogeneity was found e.g. χ^2 for heterogeneity between inbred litters of RIII females is 11.17 (df = 19) between crossbred litters of RIII females is 14.80 (df = 20), neither of which is significant.

Thus on the basis of testing the agreement of the overall ratios

of losses significant reduction in mortality occurred on crossing CBA and RIII females. Partitioning of this mortality into early (< 10 days) and later (10-17 days) is shown in the right-hand side of Table 5. When strains were pooled a very small proportion of post-implantation losses were found to occur in the later period, about 84% of losses occurring between implantation and 10 days' gestation. In the litters of RIII females in which a sizeable proportion of losses occurred in the later period it was found that reduction in mortality on crossing was affected wholly by decreasing the incidence of losses between 5 and 10 days' gestation. This applied also to the comparisons in other strains as mortality was confined mainly to the early period. Testing the ratios of early deaths to total implants between inbred and crossbred litters of RIII females gave a highly significant deviation ($\chi^2 = 12.74$, $P < 0.001$). The distribution of early losses in the two types of RIII litters is shown below.

Distributions of early deaths in inbred and crossbred litters of RIII females, i.e. numbers of dissections exhibiting each degree of early post-implantation loss.

Genotype of litter	Total no. of litters	No. of early deaths in each litter					
		0	1	2	3	4	5
Inbred	58	16	24	12	6	0	0
Crossbred	61	39	17	3	1	0	1

The striking overall decrease in mortality from 20% to 9%, on crossing resulted mainly from reduction in the number of litters in which two or three deaths occurred. The lack of heterogeneity found between litters may be explained by the few classes (0 to 3) representing individual losses.

Discussion

Significant decreases in early post-implantation mortality were found on crossing females of two strains but not on crossing females of two other strains. This was the only stage in gestation at which crossbreeding of the litter had an effect. Lyon (1959) did a similar analysis on three inbred strains and as previously stated found litter size at implantation to be maternally determined. She found significant decreases in post-implantation mortality on crossing. She also found that post-implantation deaths occurred almost exclusively in the early stage, 93% of deaths being scored as "moles".

To enable interpretation of the results found in this experiment the following comparisons were made. The degree of mortality found in crossbred litters was firstly examined by comparison of the incidences of early embryonic mortality in the four crosses (given on the right-hand side of Table 5). The incidences in crossbred litters of four types of inbred female were 7.8%, 8.5%, 9.0% and 5.5%. The ratios represented did not show significant heterogeneity ($\chi^2 = 5.2$, $P > 0.1$, $df = 3$). Lyon (1959) found no evidence of heterogeneity between incidences of embryonic mortality in crossbred litters of females of three inbred strains and the individual estimates calculated from her data are 9.8%, 7.3% and 8.0% which agree well with those above. It appears that crossbred embryos are subject to a similar degree of loss in quite a wide sample of inbred uterine environments and with respect to post-implantation survival there is little evidence to suggest that females of some inbred strains provide poorer uterine environments than others in the early post-implantation period. Secondly, on comparison of the incidences of early post-implantation mortality in inbred

litters wide differences were observed. Estimates ranged from 6.5% in JU litters to 20.2% in RIII litters (Table 5). The two ratios represented differ significantly ($\chi^2 = 29.9$, $P < 0.001$). Lyon's findings were similar.

It seems reasonable to partition early post-implantation mortality in inbred litters into two components, due to maternal and embryonic causes respectively. If these are additive components then reduction in mortality on crossing is due to elimination of that component attributable to the embryos while the maternal component is unaffected on crossing. There is some evidence that post-implantation mortality is attributable to the maternal environment. Falconer (1960) reduced litter size by selection. The reduction was subsequently found to be due to increased post-implantation mortality, the incidence of which was not reduced by outcrossing. Mortality was a property of the mothers (Falconer, 1963). What physiological causes were involved are not known. Bateman (1958) mentioned as a possible cause of early post-implantation mortality, maternal inadequacy in the development of the deciduomatal reaction at implantation. This or associated causes may be the explanation of a maternal component of post-implantation mortality.

What is responsible for the embryonic component of early post-implantation mortality? Hollander and Strong (1950) found little reduction in post-implantation mortality on crossing inbred strains. They did however record an incidence of 34% post-implantation mortality in one within-strain mating but did not include this strain in the data on the effect of crossing. They concluded that recessive lethals were of minor importance in causing embryonic death in inbred litters. The probability that recessive lethals are segregating in highly inbred stocks is extremely low unless their frequency

is maintained by recurrent mutation. Deaths due to recessive lethals are only likely to occur with an appreciable frequency when parents are related as in inbred strains. Lyon (1959) interpreted reduction in post-implantation mortality on crossing as evidence for recurrent mutation to lethal recessive genes. This hypothesis does not fit my results as no reduction in mortality was found on crossing females of the JU strain while crossing RIII females had the effect of reducing early post-implantation loss significantly from 20% in inbred litters to 9% in crossbred litters. If excess mortality in inbred litters was due to recessive lethals supplied by mutation then similar reductions in mortality should result on crossing females of any inbred strain.

Lyon offered an alternative explanation of the observed reduction in mortality on crossing. It was, that all the embryos of an inbred strain were "homozygous for recessive genes which tend to depress their ability to survive in slightly abnormal uterine environments". Reduction in litter size on inbreeding has been found to be due to reduction in the number of eggs ovulated in pigs (King & Young, 1957) and to increase in the proportion of pre-implantation losses in mice (Falconer & Roberts, 1960) i.e. through maternal components of litter size. Inbreeding depression may also act by decreasing the capacity of the young to survive. Poorer pre-natal survival of inbred young relative to crossbred young as indicated by the embryonic component of post-implantation mortality in this experiment is interpreted as inbreeding depression. This results from the fixation of recessive genes which depress the ability of foetuses to survive. Inbred strains are differentiated genetically. The gene complement of an inbred strain will be determined by the base population from which it is derived, the degree of natural selection operating in the inbreeding phase and any other factors

affecting the sampling of genes. The breeding history of most inbred strains is obscure. The JU strain used in this experiment is of relatively recent origin and was the only line surviving out of twenty on inbreeding a random-bred population and showed no decrease in litter size relative to the base population (Bowman & Falconer, 1960). Inbreeding depression in embryonic survival as represented by the reduction in the incidence of mortality on crossing is not found in this line as a result of the intense selection between lines in its derivation. It is proposed that inbreeding depression is the explanation of embryonic mortality in excess of the maternal component in inbred litters of RIII and CBA females. The occurrence of inbreeding depression will depend on the genotype of the inbred embryos and will be expected to differ between strains in degree.

The effect of heterozygosity was found to be confined to the early post-implantation stage. This is from implantation on the 5th day to approximately the 10th day of gestation. During this period the embryo depends almost wholly on its yolk-sac placenta for nutrition. Some genotypes are more prone to extinction at this stage and it is possible that stress conditions may arise under which differential mortality occurs.

Perinatal mortality has commonly been invoked to explain increases found in litter size on crossing strains of mice, yet crossing does not always increase litter size. It is therefore not a regular property of inbreds to suffer a higher incidence of mortality at this stage. In the case of C57 inbred litters perinatal mortality is invoked to explain the discrepancy between 17-day live embryo counts and the number of live young at birth. This is the only example in which this inference had to be made and direct evidence is lacking. Why should perinatal mortality occur in one strain and

not in three others in the same experiment? McLaren and Michie (1963) found that inbred litters had a significantly longer gestation period than crossbreds. It may be possible that in C57 females complications at parturition may be more common than in other inbred females. If such complications were more common in longer pregnancies it might account for a higher rate of mortality in inbreds. It is probable that decreases in post-implantation mortality and perinatal mortality are involved in the larger increases in litter size observed on crossing inbred strains, but in a hypothetical case in which the mean number of implants in inbred litters is 7 and post-implantation mortality is 35% then on crossing the expected increase is nearly 2 mice per litter, assuming 10% mortality as the maternal component. Very few observations reported in the literature exceed this.

Summary

1. Litter size on crossing inbred strains was largely under maternal determination.

2. The occurrence of heterosis depended on the strains used as female parents. Some strains showed an increase in litter size on crossing. The increase was never very large in this experiment.

3. No example of negative heterosis was found.

4. Litter size at implantation on crossing all inbred females was completely maternally determined. Ovulation rate and the incidence of pre-implantation mortality did not alter on crossing.

5. Heterosis in litter size when it occurred was due to reduction in early post-implantation mortality, except in one case where reduction in perinatal mortality was invoked.

6. Post-implantation mortality was partitioned into maternal and embryonic components. Strains did not differ in the proportion of post-implantation mortality attributable to the mother. Strains differed in the proportion of post-implantation mortality attributable to the embryos. This latter component was interpreted as inbreeding depression in early post-implantation survival.

CHAPTER IV

PRE-NATAL GROWTH

The effect of crossing inbred strains on birth weight.

Materials and Analysis.

The collective birth weight of each litter of six inbred strains and all possible crosses between them was recorded over two parities. The mean birth weight of individuals in each litter was computed. The effect of crossing was assessed by comparing the mean birth weight of crossbred young with the mean birth weight of inbred young in the same maternal environment. This was regarded as the most suitable method of comparison, as this character was largely under maternal influence. Least squares analysis, to estimate the effects of litter size, parity, and genotype on birth weight, was done for each group of litters having a common strain as female parent, i.e. each row of the six-by-six diallel table, shown in Table 1, was analysed separately. All results are presented in terms of first parity and a litter size of six, adjustment being made by correction factors estimated within common female parent groups.

The relationship between litter size and the mean birth weight of the young in the litter (in arithmetic units) was assumed to be linear. This assumption was previously tested on a large volume of data on an outbred stock. (This data was kindly lent by Dr. D.S. Falconer). Mean birth weights of 440 litters were plotted against litter size. This is shown in Fig. 2, where a linear relationship is apparent between litter

size and mean birth weight in decigrams. The straight line is the computed weighted regression of birth weight on litter size at birth. The regression coefficient was b = -0.37 ± 0.03 decigrams.

Results.

The effects of litter size and parity on mean birth weight for each of the six strains are shown in Table 6. The effect of litter size is the partial regression of birth weight on litter size, with parity held constant. The effect of parity is the increase in birth weight from first to second parity, with litter size held constant. The mean birth weight decreased as litter size increased, the effect being significant in all six strains, while it increased from first to second parity, the increase being highly significant in all strains, except KL. Table 7a shows the mean birth weights of all groups of inbred and crossbred young, adjusted to the same litter size and parity. Despite adjustment of all the means to a constant litter size, maternal effects on birth weight are apparent e.g. in the A maternal environment crossbred young (A♀ x JU♂) weighed 1.28 grams but in the reciprocal cross (JU♀ x A♂) they weighed 1.64 grams. Comparison of any crossbred group, born to JU mothers, with their reciprocal crosses illustrates this point, whereas the A strain, in particular, provides a relatively poor environment for pre-natal growth.

The effect of crossbreeding on birth weight is apparent in Table 7a. Table 7b shows the same results with each crossbred group expressed as a deviation from the inbred level of the female parent. These were estimated by least squares analysis as effects of genotypes relative to the birth weight of inbred young with litter size and parity

constant. The standard error of each difference is shown and the statistical significance of each difference is indicated in Table 7b.

The effect of crossbreeding was to increase the birth weight over the inbred level, in every case except one i.e. when JU females were crossed to CBA males, crossbred young were lighter than JU inbred young, but not significantly so. The effect of crossing on birth weight is illustrated in Fig. 3, in the form of common female parent regressions, each of which represents one maternal environment, with each cross plotted relative to the birth weight of inbreds, in the same maternal environment. No example of negative heterosis was found, none of the deviations from the inbred level being significantly negative. Differences between strains, as males on crossing, were apparent. Crossbred young of C57 and JU males ranked highest in every maternal environment, while crossbred young of CBA males ranked lowest in every maternal environment.

Conclusions and Summary.

Crossing inbred strains of mice resulted in heterosis in birth weight. Heterosis is estimated as the difference between the mean of reciprocal crosses and the mean of the two parent strains and as seen above in Table 7b this difference on crossing any two strains is positive and differs in magnitude, depending on the pair of strains crossed. The next study was designed to isolate the various factors influencing foetal weight and to attempt subsequently to draw some conclusions about heterosis in foetal growth in physiological terms.

Analysis of factors influencing foetal weight.

Introduction.

Where hybrid genotypes derive from reciprocal crosses, heterosis is estimated in two maternal environments. If the character measured is affected by the maternal environment, the study of heterosis must take account of the nature of the maternal effects. Examples of maternal effects which influenced foetal growth, as measured by birth weight, have been given.

The questions prompted by observations on birth weight, which this study attempts to answer, are:

(a) how do crossbred foetuses achieve greater birth weights than inbred foetuses in the same maternal environment?

(b) what is the cause of the negative correlation between litter size and foetal weight at birth?

(c) what is the basis of differences between reciprocal crosses in foetal weight at birth?

(d) why does foetal weight at birth increase with parity?

Before conclusions can be drawn about the nature of heterosis in foetal growth, the nature of the relationship between the foetus and its environment must firstly be defined and understood. It was by investigation of the various maternal effects on foetal growth that this understanding was sought.

Quite a vast amount of literature exists on physiological aspects of pre-natal growth, but as yet, some of the simplest questions remain unsatisfactorily answered. The experimental material in many studies was not genetically uniform and this in some cases led to the attribution of rather mystical properties to the genotype. On the other hand, studies of definite genetically determined influences on pre-natal growth have been

mainly descriptive and the physiological definitions of "genetic-environmental" interactions are long overdue.

Material and Methods.

Crossbred litters were provided by reciprocal crosses between the CBA and C57 strains and between the RIII and JU strains and inbred litters by the four within-strain matings. Females were weighed at six weeks of age and mated at eight weeks in harems of three females to one male. All matings were made within a four-month period.

Females were sacrificed 17 days after insemination as indicated by the presence of a vaginal plug. On dissection, the uterus was removed and the contents of each horn was exposed. The number of live foetuses in each horn was recorded. The collective weight of foetuses and the collective weight of placentae were recorded for each horn to the nearest centigram.

I. The effect of crossing on foetal weight and placental weight.

Analysis.

The mean weight of foetuses and the mean weight of placentae in each horn were computed. The unweighted horn mean was chosen as the weight variate in comparisons between groups and as the dependent variate in later regression analyses.

Results.

i. Heterosis in foetal weight and placental weight at 17-days' gestation.

The mean weight of foetuses and of placentae at 17 days' gestation in inbred and crossbred litters are given in Table 8. The standard error of each mean is given. It will be noted that the comparisons of inbred and crossbred groups are not biased by differences in either litter

size or size of dam between groups. Litter size was slightly greater in the crossbred group than in the inbred group of CBA, of C57 and of RIII females. This would tend to reduce rather than increase the differences in foetal and in placental weight between inbred and crossbred groups. The mean six-week weight of mothers of inbred litters was the same as that of mothers of crossbred litters in three comparisons, but JU mothers of crossbred litters were significantly heavier than those of inbred litters. This arose simply as a sampling difference, but it would be expected on the basis of later analysis to bias the weights of crossbred foetuses and placentae very slightly upwards. However, in relation to the magnitude of the differences found, due to the effect of genotype, this bias can be ignored, its effect being trivial. The mean weight of crossbred foetuses was significantly greater than that of inbred foetuses in the same maternal environment, in each of the four comparisons. Each difference is in the region of ten times its standard error, as seen in Table 8. Heterosis in foetal weight occurred in every case.

The mean weight of placentae of crossbred young was significantly greater than that of inbred young in the CBA, C57 and RIII maternal environments, respectively. What was most surprising was that placentae of crossbred young of JU females were slightly lighter than those of inbred young of JU females. This decrease was not significant, but because mothers of crossbred litters were heavier, mean placental weight of crossbred young was biased upwards. Certainly the effect of crossbreeding in this case was not to increase placental weight.

The relative effects of crossbreeding on foetal and placental weight in each maternal environment are shown in Table 9. The increase in

weight on crossing is given as a percentage of the mean weight of inbred foetuses or of inbred placentae. In three maternal environments both crossbred foetuses and crossbred placentae showed from 14% to 17% increase in weight over inbreds at 17 days' gestation. The heterosis in foetal and placental weight, as estimated by the difference between the mean of the reciprocal crosses and the mid-parent value, on crossing the CBA and C57 strains was calculated. It represented an increase of about 15% over the mid-parent value, a very sizeable effect.

(ii) Maternal effects on foetal and placental weights of crosses.

Differences between reciprocal crosses were found in foetal weight and placental weight at 17 days' gestation. These are shown in Table 10. The mean weight of crossbred foetuses and crossbred placentae were significantly greater in the CBA than in the C57 environment, and greater in the JU than in the RIII environment. As seen in Table 10 the mean weight of foetuses and of placentae in each reciprocal cross have not been corrected to a standard litter size. The difference in litter size is extremely small between the reciprocal crosses of the CBA and C57 strains. However, in the reciprocal crosses between the RIII and JU strains there is a large difference in litter size between crosses. Correction to a standard litter size in this comparison would result in a difference in mean foetal weight of about 17 centigram. between reciprocal crosses and in a difference in mean placenta weight of about 2 centigram. between reciprocal crosses. However, the correction for litter size was not necessary to show that there were significant differences in weight between reciprocal crosses which are obviously due to factors other than litter size. The computation of a correction for litter size will be seen to be a complex one and is not

presented here.

The mean weight of the mothers in each reciprocal cross is shown in Table 10. Differences in foetal weight between reciprocal crosses have often been shown to correspond positively to differences in the size of mothers representing the respective maternal environments. Thiswas true of the crosses between the RIII and JU strains but as seen by the sign of the differences between crosses involving the CBA and C57 strains this did not hold. The mean weight of crossbred foetuses and placentae was greater at 17 days' gestation in C57 females which were significantly lighter than CBA females. The slight difference in litter size tends to reduce the apparent differences in foetal and in placental weight, but this effect is small and thus has not been corrected for. It has been seen in Table 7a that the ranking of these two reciprocal crosses, for mean weight at birth, was in the reverse order. Gestation length is possibly involved in this interaction.

Conclusions and Considerations.

Differences in foetal weight at 17 days' gestation were found between genotypes in the same maternal environment, and between similar genotypes in different maternal environments. The interpretation of these differences in physiological terms is difficult. We would like to know if placental size influences foetal weight. This is relevant in deciding how heterosis in foetal weight is achieved. Huggett and Hammond (1952) state that "it would appear that the size to which the foetal placenta grows in the early stages of pregnancy may determine, other things being equal, the amount of nutrition which is at the disposal of the foetus for growth during the later stages of pregnancy." Barcroft (1944) also

subscribed to the view that placental size may be a limiting factor in foetal growth, and stressed that variation in placental size was of fundamental importance. On the basis of the first three comparisons (Tables 8 and 9), it would be difficult to decide whether the increased growth of a crossbred foetus, relative to an inbred foetus in the same maternal environment, was a property solely of the foetus, or whether it was wholly due to an increased nutritional supply by virtue of the increased placenta size. The latter hypothesis, although an improbable one, could not be disproved without the result obtained on crossing JU females which showed that crossbred foetuses were significantly heavier than inbred foetuses in the same environment even though crossbred placentae were not heavier than inbred placentae. The conclusion is that crossbred foetuses have a greater propensity for growth than inbred young in the same environment.

There still remains the intriguing question whether heterosis in placental size can contribute in ANY way to heterosis in foetal weight. The data presented do suggest that the relative effect of crossing is slightly greater on foetal weight when there is a comparable increase in placental weight (Table 9), but provide no critical evidence which would completely eliminate the possibility of the participation of the placenta in the achievement of heterosis in foetal weight.

Differences between reciprocal crosses in birth weight have usually been found to be positively correlated with differences in size between dams of the respective crosses. This situation generally held for birth weight on crossing in the previous experiment. However, on crossing the CBA and C57 strains, it was found that crossbred foetuses were heavier

in the maternal environment provided by the lighter mothers. This raises the problem of how maternal size influences foetal weight. If the effect were purely a physical one then one would not expect the ranking found.

Is placenta size involved in the mediation of reciprocal differences in foetal growth? The results in Table 10 offer no means of deciding this. It was decided to seek information about the role of the placenta and of the effect of maternal size in the simplest situation available i.e. when all mothers were genetically identical and their litters were of uniform genotype.

II. Maternal effects on foetal weight and placental weight within and between litters.

Analysis.

It was known that the weight of a foetus could be influenced, not only by the number of foetuses in the litter as a whole, but also by the number of foetuses in the same horn. This had been shown in the guinea-pig by Eckstein et al. (1955) and in the mouse by Healy et al. (1960). It had also been claimed that placental weight was determined to some extent by local effects i.e. by the number of implants in the horn independently of the number of implants in the litter as a whole (Eckstein et al., 1955). To demonstrate the existence of such effects and to help to frame a statistical analysis some relationships were plotted graphically from data provided by litters of JU females. Fig. 4 shows the relationship between the mean weight of the foetuses in the litter (i.e. the total weight of foetuses in both horns of the uterus divided by the number of foetuses in the litter as a whole) and the number of foetuses in the litter. It is clear that mean foetal weight decreases as litter size increases. On plotting the

mean weight of foetuses in the horn (i.e. the total weight of foetuses in one horn divided by the number of foetuses in the horn) against the number of foetuses in the horn, an obvious negative relationship was found (Fig. 5). However, both relationships are confounded in each of these graphs as the number of foetuses in a horn is a component part of the number of foetuses in a litter.

If the regulation of foetal growth by litter size is by systemic factors only, the distribution of individual foetuses between horns will have no influence on foetal weight i.e. foetuses in the more crowded horn of a uterus will, on average, be as heavy as foetuses in the less crowded horn. This proposition was tested by computing the difference between the mean weight of foetuses in the left horn and the mean weight of foetuses in the right horn for each of 107 litters of JU females. These differences were plotted against the differences between the number of foetuses in the left horn and the number in the right horn for all litters. This is shown in Fig. 6 which shows that foetuses were heavier in the left horn than in the right horn when this horn was less crowded than the right and vice versa. The more asymmetrical the distribution of foetuses between horns, the greater is the difference in mean weight between horns. Using differences in mean weight between horns has the effect of isolating the local influence of number of foetuses in the horn on foetal weight since the systemic influence on weight will be the same in both horns. Fig. 6 shows that foetal weight is influenced independently by number in the horn and also that this relationship can be treated as linear.

Similar analyses of placental weights showed that placental weight was not subject to a local effect of any great magnitude. Fig. 8

illustrates this and mean placental weight, if anything, is greater in the more crowded horn. Fig. 7 shows that the mean weight of placentae in the litter decreases as litter size increases. No obvious relationship was found on plotting the mean weight of placentae in the horn against number in the horn.

Multiple regression methods were used to determine the magnitude of systemic and local effects on foetal and placental weight. The results were obtained in the form, $Y = K + b_1X_1 + b_2X_2$ ------, where Y is the mean weight of foetuses or placentae in one horn (in grams), X_1 is the number of implants in the horn and X_2 is the number of implants in the litter as a whole. K is a constant determined by substituting the mean value of the different variates and solving a regression equation of the form

$$y = b_1x_1 + b_2x_2 + \text{------},$$

where the zero of each variate is taken at its mean (Woolf, 1951). The partial regression coefficients b_1 and b_2 estimate the local and systemic effects, respectively. Each analysis was confined to litters of the same genotype i.e. eight separate groups of litters, four crossbred and four inbred, were analysed separately. The six-week weight of mothers was included in each analysis, firstly, because litter size is positively correlated with the weight of the mother (Falconer, 1955) and secondly, to determine the influence of the weight of the mother on foetal and placental weights within strains. It is included as the third independent variate (X_3) in each analysis.

Results.

The local and systemic effects of litter size on foetal weight at 17 days' gestation, as measured by the partial regressions of the mean

weight of foetuses in the horn on number in the horn and number in the litter respectively, are shown in Table 11 for each of eight series of litters. The local effect was negative in every case and was significant in five of the eight series. The systemic effect was negative in seven series, significantly so in three cases. The general situation is that the weight of a foetus is determined independently by the number of foetuses in the litter as a whole and by the number of foetuses in the same horn. These effects are additive and thus, in simple terms, these findings mean that an additional foetus in one horn will reduce the weight of foetuses in that horn by an amount equal to the sum of the local and systemic effects, whereas it will reduce the weight of foetuses in the opposite horn by an amount equal to the systemic effect only. The effective reduction in weight of foetuses in the same horn will be about twice as great as that in weight of foetuses in the opposite horn, as local and systemic effects are of the same order of magnitude.

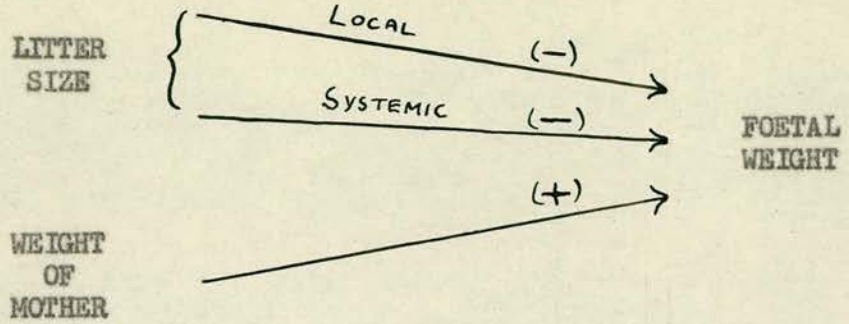
The local and systemic effects of litter size on placental weight are given in Table 12. The local effect was positive in all series but only significant in one case. The systemic effect was negative in all cases and highly significant in most. Fig. 8 showed that local effects on placental weight in JU litters were virtually absent. However, it must be concluded that generally placentae in the more crowded horn of a uterus will be slightly heavier than those in the less crowded horn since the effect is positive in eight out of eight cases. This is extremely unlikely to occur by chance although individually, the regression coefficients are not significantly different from zero. Whatever the biological explanation of this situation is, one fact emerges, that is, that the local effect on

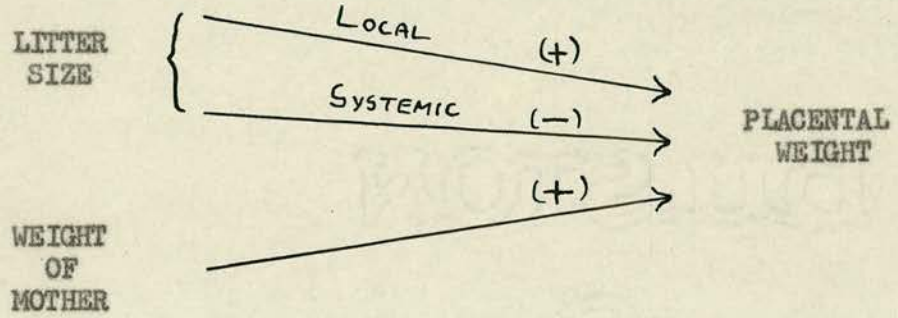
foetal growth is not mediated by the size of the placenta.

The effect of the six-week weight of the mother on foetal weight, as measured by the partial regression of the mean weight of foetuses in the horn on the weight of the mother, is shown in Table 11. The effect was positive in all eight cases and significantly so in four. The effect of the weight of the mother on placental weight is shown in Table 12. The effect was positive in six series, significantly so in four and negative and non-significant in two cases. Females in these eight cases were mated at eight weeks of age. The independent variate in estimating the effect of weight of mother on foetal weight and placental weight was the weight at six weeks of age. It was decided to analyse a check series of JU inbred litters in which females were weighed at insemination. The results, shown in Table 13, agree well with former results shown in Tables 11 and 12 where six-week weight was used as the independent variate (X_3).

Heavier mothers tend to have heavier foetuses at 17 days' gestation in all series analysed. This effect was statistically significant in five cases. A similar generalization may be made about placental weight. Each analysis was applied to the products of gestation of females of similar genotype and statements about between-litter maternal effects on foetal and placental weights refer to differences between females of the same strain. That these statements cannot be extended to litters of females of different strains has already been made clear.

The factors affecting foetal weight and placental weight are summarised diagramatically below.

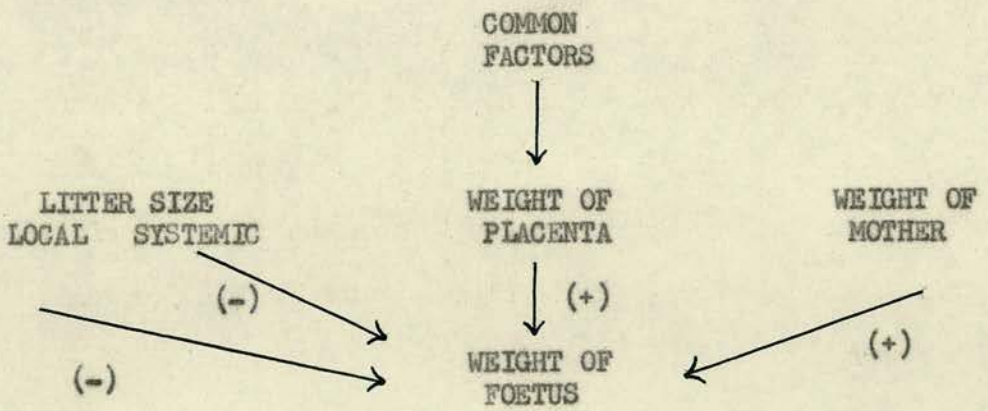




To what extent are the systemic effect of litter size and the effect of the mother's weight on foetal growth, a reflection of similar effects on placental weight? Are foetuses of large litters smaller because they have smaller placentae which limit the supply of food available to foetuses later in gestation? An attempt to answer these questions was made by including placental weight as the fourth independent variate in the multiple regression of foetal weight on number in the horn (X_1), number in the litter (X_2), weight of the mother (X_3) and placental weight (X_4). The effects of litter size and mother's weight on foetal growth were estimated with placental weight held constant, to determine if these effects operated independently of the size of the placenta. The results are shown in Table 14. As expected, the local effect of number in the horn on foetal weight was

independent of placental size. The magnitude of this effect (b_1) was not decreased by holding placental weight constant and placental size apparently played no part in the mediation of this effect. The systemic effect of the number in the litter (b_2) and the effect of mother's weight on foetal weight (b_3), were not wholly independent of placental size, as seen by comparison of the magnitude of these effects in Tables 11 and 14. Nevertheless, with placental weight held constant, the systemic effect of litter size was <u>negative</u> in seven out of eight series, significantly so in one case. Similarly, the effect of mother's weight, acting independently of placental size, was <u>positive</u> in <u>all</u> series, significantly so in two cases. The conclusion is that these two effects on foetal weight were not mediated, to a great degree, by the size of the placenta.

There remains the partial regression of foetal weight on placental weight. This was positive in all series, significantly so in four cases. The independent effects on foetal weight are shown diagramatically below.



The interpretation of the relationship between placental weight and foetal weight is difficult. The positive correlation between them could have arisen due to the influence of one or more factors which favoured or retarded the growth of both. A causal relationship between placental weight and embryo weight cannot be concluded. This is indicated in the diagram.

III Maternal effects on foetal weight and placental weight between parities.

Analysis.

The mean birth weight of litters of standard size increased significantly from first to second parity. Data on foetal weight and placental weight of inbred JU litters were available for first parity. Similarly data were collected on foetal and placental weight of two series of JU litters in second parity. Dissections were made 17 days after the detection of a post-partum plug in the first series, in which first litters were removed at birth. (Concurrent lactation was known to decrease foetal survival in this strain). Dissections in the second series were made 17 days after the detection of a post-weaning plug. Thus, in neither case was lactation concurrent with gestation of second litters.

Results.

The mean foetal weights and mean placental weights in first litters and in all groups of second litters are shown in Table 15. Placental weight increased significantly in second parity, whereas foetal weight decreased, significantly so in Series (b). The differences between groups in litter size were unimportant firstly, because they were small and secondly, because litter size influences both foetal and placental weight

in the same direction whereas differences in foetal weight and placental weights in Table 15 are of opposite sign. Sugiyama (1961) in a much larger experiment than this found that mean placental weight at 18 1/2 days in the mouse increased significantly with parity but that mean foetal weight did not increase. The effect of parity on foetal weight at birth must be attributed to an increase in gestation length. Increased gestation length in later parities has been observed in many species (for review, see McKeown and MacMahon, 1956).

Discussion.

Genetic and environmental effects on foetal and placental weight have been observed in this study. Before considering the implications of the observed effect of crossing on foetal growth, the causes of environmental variation will be considered. It was found, as in many other studies, that foetal weight decreased as litter size increased. It may be said at this point that very little evidence exists which helps even to speculate on the possible nature of this effect. If the physiological basis of this influence were known, some light might be shed on the nature of the factors causing differences between reciprocal crosses in foetal weight. Indirect evidence found in this and other studies provides some guidance to future investigation of these problems.

The original explanation, offered to explain the reduction in mean foetal weight with increase in litter size in polytocous species, was one of nutritional limitation. The evidence, which was based mostly on observations of variation in foetal growth of the rabbit, was reviewed by Hammond and Marshall (1952). The inhibition of foetal growth in large

litters was attributed "to the limitation of some nutritional substance in the blood supply of the mother". No evidence of local effects being found, the hypothesis inferred that the growth-retarding effect of increasing litter size was wholly systemic in action. The results presented above show clearly that local effects were as important as systemic effects on foetal growth in the mouse. If competition for a specific nutrient is to be invoked to describe this situation, then the original hypothesis must be modified to account for differential growth in the two horns, according to degree of crowding. It is difficult to see how Hammond and Marshall's hypothesis could allow individuals in one horn to extract more nutrients from a common pool than those in the other horn.

An alternative explanation, to that of competition for a limited supply of some nutritional substance, was put forward by Eckstein et al. (1955). They proposed a haemodynamic model to explain observed variation in foetal weight in the guinea-pig. A systemic effect and a less marked local effect on foetal weight, both independent of placental size, in the guinea-pig, were suggested as evidence for limitation of blood supply to the uterus. The authors proposed that differences in blood-pressure between horns, caused by interposition of different numbers of placentae in the two lateral uterine circulations, were responsible for the local effect on foetal weight. On the premises that each placenta represented a low resistance to blood flow in a uterine artery and that the pressure at which blood was supplied to each placenta was inversely related to the number of placentae in the same horn, they suggested that differential blood pressure between horns, having different numbers of foetuses, would

result in local effects as observed. Thus blood supplied to the more crowded horn would be at a lower pressure and consequently have a lower rate of flow. To accommodate systemic effects in this explanation they postulated that reduction in blood pressure in one uterine artery was likely to be transmitted in part to the contralateral one.

The pattern of variation in foetal weight in the mouse has been thoroughly investigated by Healy et al. (1960). These authors found little support for the theory of competition for a common nutritional pool. Superovulated adolescent females were found to yield full-term foetuses as heavy as those of a parallel series of superpregnant adults. Their findings are reviewed by McLaren and Michie (1960) in terms of the haemodynamic hypothesis. The existence of substantial local effects on foetal growth, and of local and systemic effects of embryos not surviving to late pregnancy, fitted well with the haemodynamic hypothesis. Probably the most convincing demonstration, quoted by McLaren and Michie (1960), of the involvement of differential blood flow in causing intra-uterine variation, was their investigation of within-horn variation (McLaren and Michie, 1959). The weights of individual foetuses of super-pregnant females were recorded. The distribution of foetal weight formed a U-curve from the ovarian to the vaginal end of the uterine horn. Each horn of a mouse uterus is supplied with blood through offshoots of a main loop artery fed from the abdominal aorta at the top and from the iliac artery at the bottom. This pattern of variation was interpreted as consistent with the expected gradient in blood-pressure in a series of offshoots from the main uterine artery.

My results are more likely to be accommodated in terms of the

haemodynamic hypothesis but due to the lack of direct experimental evidence on the dynamics of blood flow, explanation of variation both in foetal and placental weight involves many assumptions. Some relevant points emerge from my results, particularly with respect to the correlation between placental size and foetal size. This correlation has been observed in many species in a large number of separate studies and has always been a source of confusion. On intuitive grounds, one might suppose that the area available for molecular transfusion between the maternal and the foetal circulations might be related to the "size" of the placenta but in view of the multiplicity of factors involved in placental exchange, gross oversimplifications should be avoided. The patterns of between-horn variation in foetal weight and in placental weight in my experiment provided a striking contrast, and demonstrated that factors other than placental "size" must be considered in explaining environmental influences on the rate of foetal growth. The "size" of the placenta is one thing, the amount of blood which is circulated through it is another.

A highly relevant finding, with regard to the interpretation of variation in foetal growth, is that of Rodbard and Katz (1944). They found that maternal blood pressure in normotensive dogs tended to fall in the latter part of pregnancy. This was more marked in dogs rendered hypertensive experimentally. The degree of reduction in pressure was apparently effected by the size of the litter, more marked reductions occurring in bitches with large litters. Although the experiment was on a small scale, their evidence strongly suggests why mean foetal weight and litter size are negatively correlated. The amount of maternal blood which is circulated

through the vascular bed of the placenta depends on the pressure difference between the arterial and venous ends. The effective placental arterial blood pressure will be highly correlated with the blood pressure in the arteries entering the uterus until near term, intrauterine resistance being at a low level. Thus, reduction in maternal blood pressure will be reflected in a correlated reduction in the volume of blood circulated through the placenta. The systemic effect of litter size on foetal weight, which was independent of placental size, was not very large in my experiment but it was negative in seven series. It may well have been a reflection of the effect of litter size on maternal blood pressure, foetuses in large litters receiving relatively less blood than those in smaller litters, in the latter part of pregnancy. Could the systemic effect of litter size on placental weight be explained on the same basis? This is very difficult to say in view of the lack of information about the coincidence of growth in the placenta of the mouse and maternal blood pressure fluctuations.

There are several possible explanations of the local effect of number in the horn on foetal weight, in haemodynamic terms, but no evidence exists to support any of these. As the demand on the general circulation for blood increases when the maternal placental circulations are increasing in size, each main uterine artery must supply more blood. The greater the number of placentae in the horn, the greater the volume of blood in the vascular system of that horn. If there is an upper limit to the volume of blood that can be shunted through the main artery of a horn, then the rate of flow of blood through each placenta will decrease as the number of placentae in each horn increases. This may explain why foetuses in a crowded horn are at a relative disadvantage and grow at a lower rate.

Reynolds (1949) suggested that the greater total uterine distention resulting from a larger number of smaller individuals may limit the nutrition to each foetus by an effect on the available maternal blood supply. This could also be the explanation of the local effect on foetal growth. There is no evidence available which can decide whether the local effect on foetal growth is caused by blood pressure differences as suggested by Eckstein et al. (1955) or by physical effects determined by the capacity of the uterine circulation.

The finding of a small positive local effect on placental weight is puzzling. No explanation is obvious. Indeed, in view of the magnitude of the systemic effect on placental weight, it is unlikely that any of the foregoing considerations are adequate to explain either of these effects. Nevertheless, the obviously complicated nature of the causes of variation in foetal and placental growth is more likely to be explained ultimately in haemodynamic terms, rather than on a simple nutritional hypothesis.

It is tempting to speculate on the origin of the effect of mother's size on foetal and placental weight. It is possible that the effect of size results from a positive correlation between the vascular capacity of the mother and her size. A larger body size might result in a larger vascular system which could supply relatively greater amounts of blood to the developing foetuses. Are there genetically determined differences between strains in "vascular capacity"? Froud (1959) found differences between strains of mice in some arterial features which he states "demonstrate this arterial variation to be under genetic control". These studies applied to variation in point of origin of individual vessels but

from the haemodynamic aspect, variation in arterial caliber, total blood volume, and other features associated with rate of blood flow are of greater interest. Studies along these lines might help in understanding why differences between reciprocal crosses occurred in foetal weight, which as seen from my results, do not always reflect the differences between the size of the maternal strains.

As to the physiological means by which heterosis in foetal weight was achieved, it would seem that placental heterosis was of little importance. We have seen that in one case crossbred foetuses attained greater weights at 17 days' gestation than inbred foetuses in the same maternal environment, with no relative increase in placental size. It has also been shown that placental size played little part in the determination of foetal weight, as demonstrated by the local and systemic effects of litter size and the effect of parity, all independent of placental size. Gates et al. (1961) have shown that genetic differences in foetal growth are expressed very early in development. Crossbred foetuses which weighed about 20% more than inbred foetuses near term in the same environment, had achieved a considerably greater degree of development near the time of implantation. The latter was determined from counts of nuclei in squash preparations. Crossbred foetuses obviously have a greater propensity for growth than inbred foetuses, in the same environment. There is no evidence, however, on how this is achieved. The increased demands of crossbred foetuses are met by the maternal environment. It may be worth while to comment on the theory of competition for a specific nutrient in this context. If foetal growth in larger litters is retarded because there is a limited supply of some essential nutrient, it must follow

that crossbred foetuses have a lower requirement for this substance. As we shall see, this is not always the case. Bogart et al. (1958) found that crossbred young grew more slowly than inbred young, in an environment in which biotin and folic acid were limited in supply. Prabhu and Robertson (1961) found that inbred lines of Drosophila showed less reduction in body size below the wild yeast level than either crosses or the wild population, when media deficient in fructose or RNA were used. They suggested that the inherently faster growth of non-inbred individuals may create a relatively greater requirement for a high concentration of certain essential nutrients. The theory of competition for a specific nutrient as the basis for variation in foetal growth is hardly compatible with the very marked heterosis observed in pre-natal growth in this experiment.

Summary.

1. Heterosis in birth weight was found on crossing inbred strains of mice.

2. Birth weight was negatively correlated with litter size.

3. Birth weight was greater in second parity than in first.

4. Foetal weight and placental weight at 17 days' gestation showed heterosis on crossing.

5. Maternal effects on foetal weight and placental weight occurred. Differences between reciprocal crosses were significant.

6. Within strain analysis of variation in foetal and placental weight showed that (i) foetal weight was determined independently by number of foetuses in the horn (local effect), by the number of foetuses in the litter (systemic effect) and by the weight of the mother.

(ii) Placental weight was determined independently by the number in the horn (small local effect), by the number in the litter (systemic effect) and by the weight of the mother.

7. Effects on foetal weight were largely independent of placental size.
8. Placental weight increased in second parity. Foetal weight did not. The results are discussed and an interpretation of maternal effects is attempted in terms of a haemodynamic hypothesis.
9. No example of negative heterosis in pre-natal growth was found.

CHAPTER V

POST-NATAL GROWTH

The effect of crossing inbred strains of mice on weaning weight.

Introduction.

Mason, Nicholson, Bogart and Krueger (1960), on crossing four strains of mice, found "that all significant differences from parental means for characters concerning growth and body weight were negatively heterotic". This the authors regarded as resulting from the fact that the four strains "displayed conflicting physiological patterns" which "were mostly additive in their inheritance". On crossing, this resulted in "a conflict between the physiology of the mother and the genotype of her crossbred offspring". In an earlier paper, Bogart et al. (1958) described one such physiological incompatibility in detail. Crossbred young reared by females of one particular strain showed symptoms of vitamin deficiency about eight days after birth. Injection of the mothers or of the crossbred young, with a solution of biotin and folic acid, resulted in increased survival and normal growth of the young. That a maternal effect was responsible for the incompatibility was shown by the fact that it did not occur in the reciprocal cross, and that treatment of the mothers was effective. The physiological explanation of this situation was based on observations made on the relative survival times of individuals from each of the strains and crosses in asphyxiation tests. Some strains expired sooner than others. Crossbred offspring usually survived asphyxiation for shorter periods than did the parent strains (Mason et al., 1956). The hypothesis offered to

explain the maternal-offspring incompatibility mentioned assumed that asphyxiation time was inversely related to thyroid activity. The maternal strain involved was, on this basis, "hypothyroid". The authors suggested that pure-strain offspring survived when reared by these mothers because they too were "hypothyroid", but that crossbred offspring, being "hyper-thyroid", required more vitamin than the mothers could supply and therefore showed deficiency symptoms. This explanation embodied two further assumptions, firstly that the hypothyroid state led to "a low rate of assimilation of biotin and folic acid" and secondly, that thyroid activity was correlated with vitamin requirement. Due to the lack of evidence to support any of these assumptions the physiological explanation is questionable. Whether in fact the thyroid was implicated is only of secondary importance, since intergeneration incompatibilities were actually found, and occurred to such a degree that crossbred young were at a serious disadvantage relative to inbred young, in certain maternal environments. Several other less severe instances of the same type of situation were reported in the one experiment, in which four strains of mice were crossed. This resulted on final analysis in "predominance of negative heterosis" in characters related to growth (Mason et al., 1960).

Bowman (1962), in an experiment on recurrent selection for litter size in mice, encountered an apparent maternal-offspring incompatibility. An inbred line was used as a "tester" stock. Males of an outbred stock were mated to a sample of inbred females and selection of males was based on the mean litter size of a half-sib group of testcross daughters. No heterosis in the litter size of F_1 females was found. The mean litter size of crossbred females was lower than that of either parent

in three cycles of selection and in the fourth and last cycle, did not reach the mid-parent value. In the inbred maternal environment, crossbred young were inferior to inbred young in neo-natal viability, survival from birth to weaning and in mean weaning weight (Bowman, 1958). Crossbreds were as heavy as inbreds at six weeks of age, yet as stated, females on intercrossing had smaller litters than inbred controls. That a maternal effect was responsible for this situation was shown by the reciprocal cross, in which crossbreds in the outbred maternal environment were equal, in all respects, to the better parent. The inbred tester stock was the JU strain and this was one of the six strains crossed in this experiment.

It has been shown in my experiment that no apparent maternal-offspring incompatibility was encountered in the first two characters studied, namely litter size and pre-natal growth. It was possible that one such interaction might be found in post-natal growth and thus the following procedure was adopted.

Analysis.

The individual weights of all young of six inbred strains and all possible crosses were recorded at 21 days of age (i.e. at weaning). The mean weight of individuals in each litter was computed and this was the variate used in analyses. The method of analysis used was the same to that employed for birth weight i.e. crossbred young were compared to inbred young in the same maternal environment. Least squares analyses were used to estimate the effects of genotype relative to the inbred level, with litter size and parity held constant, in each of the six maternal environments. "Litter size" in this context was the number of young alive at weaning. The assumption in

these analyses of a linear relationship between mean weaning weight of the litter and the number weaned was previously tested on a large volume of data on an outbred stock (kindly lent by Dr. D.S. Falconer). This is shown in Fig. 9 where a linear relationship is apparent over a wide range of litter sizes.

Results.

The weaning weight of crossbred young relative to that of inbred young is shown in Fig. 10 for each maternal environment. Litter size in all cases was adjusted to six. The results are presented in terms of first parity. It can be seen from Table 16a and Fig. 10 that maternal determination of this character was not as marked as in the case of birth weight. There was some correlation between the performance of crossbreds and the performance of the strain as male parent in weaning weight. Crossbred young of JU males ranked highest in all five maternal environments. The differences between the mean weaning weights of five crossbred groups and that of the inbred group in each environment are shown in Table 16b. The standard errors of these differences were computed and the significance of each difference is indicated. In no instance were crossbred young significantly lighter than inbred young in the same maternal environment. One feature was outstanding. Crossbred young of JU males ranked high in five environments but in the JU maternal environment none of five crossbred groups was significantly different in weaning weight from the inbred (JU) group, and three of the deviations were, in fact, negative. It must be appreciated, however, that these inbreds had a high mean weaning weight. In view of the fact that this was the strain used as the inbred "tester" by

Bowman (1958), further investigation was carried out to ascertain if the growth of crossbred young, in the JU maternal environment, was restricted to the inbred level by any interaction between crossbred young and maternal environment.

A number of vitamins, among them riboflavin, niacin and pyridoxine, were included in the stock diet in this laboratory from 1960 onwards (see Table 17 for details). It was suspected that their addition might explain why the result of Bowman (1958) was not repeated in this experiment. This former result, however, had not indicated an incompatibility of any great magnitude and thus a low-plane diet was chosen in subsequent comparison of post-natal growth of inbred and crossbred litters of JU females.

SUBSIDIARY EXPERIMENT

The effect of different post-natal environments on pre-weaning growth of crossbred and inbred litters of JU females.

Introduction.

The object of this study was to determine why crossbred young of JU females showed no increase in the rate of post-natal growth over that of inbred young. Was it because heterozygosity conferred no advantage in growth potential or was it because crossbred young were retarded by some aspect of the maternal environment? If the latter were true, comparison of crossbred and inbred genotypes in different environments should expose genetic-environment interaction.

Materials and Method.

Two large samples of virgin JU females were mated to C57 and JU males to provide crossbred and inbred litters. Matings were maintained on

the standard stock diet (used in previous experiments) until litters were born. On giving birth, some mothers of both inbred and crossbred litters were transferred to a low-plane diet consisting of oats and maize (ad libidum), while others in both samples remained on the standard stock diet as controls. Some crossbred and inbred litters were removed at birth and fostered on uniparous outbred foster mothers. Crossbred and inbred litters, of JU females, were thus compared contemporaneously in the following three post-natal maternal environments:

(i) outbred females on standard diet (designated "Fostered");

(ii) inbred (JU) females on standard diet (designated "Control");

(iii) inbred (JU) females on oats and maize (designated "Minimal").

Approximately 30 inbred litters and 30 crossbred litters were compared in each environment.

Results.

The mean litter size and the mean weaning weight of each group of crossbred and inbred litters in each environment are shown in Table 15. There was no significant difference between the number of mice weaned in crossbred litters and inbred litters in the same environment and thus no adjustment was made for small differences between groups in litter size. The effect of the plane of nutrition on the rate of post-natal growth of both crossbred and inbred young is apparent in Table 18. Young in the better environment, provided by outbred females, reached more than twice the weight attained by young of similar genotype, in the poor environment provided by JU mothers on oats and maize.

There was an obvious genotype-environment interaction in weaning

weight. Crossbreds were significantly heavier at weaning than inbreds in the best environment, provided by outbred females. Crossbreds were slightly heavier than inbreds, but not significantly so, in the control JU environment, as found in the previous experiment. Crossbreds, however, showed no superiority over inbreds in post-natal growth in the poorest environment, provided by the JU mothers, on oats and maize.

Discussion.

No example of maternal-offspring incompatibility was found on crossing six strains. A genotype-environment interaction was exposed in a subsidiary experiment. Crossbred young were superior in the best environment to inbred young yet no evidence of superiority in rate of growth was shown in the poorer environments. Genotype-environment interactions of a lesser degree have been shown in Drosophila by Prabhu and Robertson (1961). By reducing the concentration of specific nutrients in the diet, relatively greater reductions in body size, below that on optimal diet, were observed in crosses than in inbreds. Dickinson et al. (1962), on comparing the pre-natal growth of Lincoln and Welsh sheep in three different maternal environments, found that as the maternal environment became poorer, genotypic differences remained distinct but decreased in magnitude. All these observations suggest that individuals, which have an inherently faster rate of growth, may require a relatively greater available supply of essential nutrients. The retardation of the post-natal growth of crossbred young to the inbred level in the JU maternal environment may be due to a deficiency of one or several nutrients in the milk supply of JU mothers. The interaction is not a marked one by comparison with those found by Bogart et al. (1958)

and further study was not attempted on this problem.

This entire study yielded no information about maternal-offspring incompatibilities. They must be regarded as atypical situations and their exposure may well depend on the nature of the nutritional environment in which breeding is carried out.

APPENDIX

A physiological study of the effect of concurrent lactation in the JU strain.

The effect of concurrent lactation on the pre-natal viability of second litters in an inbred strain of mice

INTRODUCTION

The variation of litter size with parity in mice is well known. The size of the first litter is submaximal and litter size increases until a maximum is attained in the third or fourth parity. A "plateau" period follows during which litter size is greatest for a variable number of subsequent litters. Later in the reproductive life litter size declines. Biggars et al. (1962) have surveyed published data on the lifetime reproductive performance in polytocous mammals and have found that this pattern is common to mice and rats and is true to some degree of pigs. The initial increase in litter size is attributable to an increase in the number of eggs shed in mice (MacDowell & Lord, 1927, quoted by Biggars et al. (1962)) and in pigs (Hammond, 1914; Perry, 1954).

In the course of an experiment on the effect of crossing inbred strains of mice on fertility, McCarthy (1963) found that the litter size of one strain was maximal in first parity, second litters being significantly smaller than first. This strain (JU) was a listed inbred strain (Standardized Nomenclature for Inbred Strains of Mice, 1960) and had been maintained in this laboratory for a number of years. The stock was maintained as full-sib matings, caged in pairs, so that males were continuously present. Records of litter size were analysed for the period 1960-62. All females, having at least two litters, were included in the analysis. As the stock diet had been altered during this period, comparisons were made between the means of the size of first and second litters within diet-periods.

The results are shown in Table 19, together with a similar comparison for two groups of independently maintained JU stocks which were recorded in 1962 (designated stock 1 and 2 in Table 19). Second litters were significantly smaller in all comparisons. Although litter size was apparently influenced by diet, the ranking of litter size with respect to parity did not change.

Bruce and East (1956) studied the effect of concurrent lactation on litter size in various non-inbred stocks and found no effect as drastic as that described above, litter size being virtually unaffected by concurrent lactation. Roberts (1956) also found no effect of concurrent lactation on litter size in two outbred stocks. This investigation was carried out to determine what factors were involved in the causation of this unusual situation. It was found that concurrent suckling of first litters affected the prenatal viability of the second litters. The latter part of this paper deals with the investigation of this specific effect, which was hormonal in nature.

I. Preliminary analysis of records.

Records were available on second litters of four groups of females (Table 19). An analysis of variance of the size of second litters was done to ascertain if there was significant variation between groups. The result is shown in Table 20. The size of second litters did not vary between groups and further analysis of second litters was conducted on the pooled data.

Second litters were classified according to the period, in days, elapsing between the birth of the first litter and the birth of the second

litter. A bi-modal distribution was obtained, second litters being born between 20 to 38 days or between 40 to 100 days with modes at 26 and 45 days respectively. Second litters, born from 20 to 35 days and from 40 to 80 days after the birth of the first litters, were classified as "early" and "late" respectively, 74 litters comprising the former and 53 litters the latter class. It was assumed that "early" second litters were conceived as a result of post-partum insemination and that litters born after an interval of 40 days resulted from insemination occurring after the termination of the suckling period, i.e. 21 days after the birth of first litters. If insemination of females occurred during lactation, then litters resulting from such inseminations would be scored as "early". The available literature on the effect of lactation on oestrus in the mouse (e.g. Bruce and East, 1956) indicated that when large litters were suckled, oestrus was suppressed. In later experiments described below it was found that in this strain insemination occurred in the post-partum period but not during the suckling period, i.e. oestrus occurred shortly after parturition and did not recur until the litter was weaned 21 days later. The variable interval between first and second parturitions in the early class was a reflection of delayed implantation caused by concurrent suckling as had been found in many other studies (e.g. Enzmann et al., 1932). The mean litter size of "early" and of "late" second litters were compared. The means of the two respective groups were 6.5 ± 0.3 and 8.2 ± 0.4, the difference between them, 1.5 ± 0.5, being significant at the one percent level. Second litters, which had been gestated with concurrent lactation, showed a significant reduction in

litter size relative to second litters which were conceived after lactation had ceased. The following experiment was designed to find out at which stage, or stages, litter size was reduced in the presence of lactation.

II. The effect of concurrent lactation on the components of litter size in second litters of JU females.

MATERIAL AND METHODS

Virgin JU females were mated at eight weeks of age, in single-pair matings, with males of the same strain. Births were recorded daily. On the birth of the first litter, the occurrence of post-partum mating was determined by the presence of a vaginal plug. The frequency of post-partum mating was extremely high. Females which did not mate in post-partum oestrus were discarded. Males were continuously present in this experiment.

Mothers were allowed to suckle their litters for a period of 21 days, after which litters were weaned and weighed. After the weaning of first litters females were examined regularly and those which were pregnant were dissected. Pregnancy was determined by palpation of the uterus. About 50% of all females which had mated in post-partum oestrus and had suckled their litters became pregnant to that mating, the others remating after the first litters were weaned. This was acertained by searching for vaginal plugs. Thus it was assured that in one group of females the products of gestation resulted from post-partum insemination and that a control group was available in which gestation had proceeded without the complication of lactation. This latter group was dissected at 17 days' gestation as indicated by the presence of a vaginal plug in the post- weaning

period. At dissection, counts were made of (1) the number of corpora lutea, as a measure of the number of eggs ovulated, (2) the number of live embryos, and (3) the number of dead implants. Dead implants were classified on a morphological basis in order to ascertain the stage of gestation at which death occurred. The difference between (1) and the total number of implants, given by the sum of (2) and (3), indicated pre-implantation losses. Post-implantation losses were indicated by (3).

The classification of post-implantation losses was based on the appearance of the dead implants. Those represented by "moles" were scored as "early" deaths. Any implant which was represented by an undersized placenta without a live embryo was scored as a "middle" death. Dead embryos of recognisable bodily form were scored as "late" deaths.

On dissection of females, which had concurrently suckled their first litters, the age of live embryos at dissection was ascertained by reference to the development of external features (Grüneberg, 1943). The difference between the interval in days, from post-partum mating to dissection, and the age of live embryos at dissection, provided an estimate of the delay in implantation caused by lactation. No evidence was found that mating occurred during the suckling period, on searching for vaginal plugs in that period, so that this approximation of the duration of delay in implantation was valid.

RESULTS

Delay in implantation

Table 21 shows the numbers of pregnant females dissected in the lactating and non-lactating groups, the age of live embryos on dissection

and the mean delay in implantation in the lactating group. As the delay in implantation in lactating females was unpredictable, the age of embryos at dissection in this group varied, the mean age being about 14 days. Some females, which were not pregnant, were dissected in error but these were not included in the calculations.

Implantation was delayed in all females which were concurrently lactating. Table 22 shows the distribution of the duration of delay in implantation and the mean number of young suckled during those pregnancies. The number of young weaned was more than five in all litters except one and although it would appear from Table 22 that a slight correlation might exist between the number suckled and the delay in implantation, it is evident that little variation existed in the number of young suckled whereas there was wide variation in the duration of delay in implantation.

The components of litter size.

Table 23 (first two rows) shows the number of corpora lutea, live young and the percentage mortality estimated from dissections of the two groups of females. All females had mated in post-partum oestrus so that the 36 females with "uncomplicated" pregnancies had lost whole litters during the suckling period, either by failure of all the blastocysts to implant, or by later abortion of the whole litter. In either case re-mating would occur as was observed in the control groups. The confirmation and significance of whole litter losses will be presented later.

The number of eggs shed as indicated by corpora lutea counts was the same in both groups. The number of live young in second litters, found at dissection, in lactating females was much lower than in non-lactating

females; a difference of 3.2 which was highly significant ($p < 0.001$). The proportion of pre-implantation loss was less in the lactating females so that the reduction in the number of live young in the lactation group must have occurred in the post-implantation stage. This is the most striking feature of the data in Table 23. Post-implantation deaths occurred with an extremely high frequency in concurrently lactating females, amounting to almost 50% of the total implantations. This estimate is biased downwards, firstly because dissections were made earlier than in the control group, and secondly, because no account was taken of whole-litter losses occurring in the post-implantation period. The incidence of post-implantation deaths in the non-lactating females was not appreciably higher than estimates of such losses found in first pregnancies of four inbred stocks (McCarthy, 1963).

Direct confirmation was obtained that lactation was responsible for the increased post-implantation mortality in litters, which were gestated with concurrent lactation. The first litters of 30 females were removed at birth. Post-partum mating occurred in every case and all females were dissected 17 days later. The number of females pregnant at 17 days' gestation, the components of litter size and the estimates of mortality are shown in the third row of Table 23. Nearly all these females became pregnant to post-partum insemination. It was already observed that when lactation occurred in 79 females which had mated in post-partum oestrus, only 54% actually became pregnant to that insemination. This indicated that whole-litter losses occurred frequently when lactation was concurrent with gestation. When first litters were

removed at birth the mean number of live young in second litters at dissection was 9.1 ± 0.41 which was significantly greater than 5.4 ± 0.38 live young in the lactating group (diff. = 3.7, $p < 0.001$). Post-implantation mortality was 17.0%.

It was evident that concurrent lactation in JU females reduced the size of second litters by increasing post-implantation mortality. Concurrent lactation also decreased the proportion of females which became pregnant to post-partum insemination. This was interpreted as an increase in the frequency of whole-litter losses.

Post-implantation mortality.

The partitioning of post-implantation deaths, according to the stage of development reached by dead implants, in second pregnancies of lactating females and of two groups of non-lactating females, is shown in Table 24. The incidence of loss in litters of lactating females was increased in all post-implantation stages. The most divergent estimates for lactating and non-lactating females were obtained in the "middle" period, i.e. the proportion of losses in lactating females, which were represented at dissection by an undersized placenta, with no identifiable embryo attached, was greater in concurrently lactating females. About 26% of implantations in lactating females died during the "middle" period, as compared to about 2% in non-lactating females. Although no histological study was attempted, it was apparent at autopsy that "middle" deaths (i.e. occurring from 10 to 12 days after implantation) were associated with placental abnormalities. Breakdown of the placenta occurred at an early stage and decidual necrosis was observed on dissection of the placenta

under a binocular microscope. Uteri of lactating females at autopsy were usually lined with extravasated blood.

The observations in many autopsies were typical of results of ovariectomy after implantation in rodents, e.g. Huggett and Pritchard (1945). Consequently hormone therapy was applied in an attempt to reduce post-implantation mortality in second litters of concurrently lactating females.

III The effect of progesterone on pre-natal mortality in second litters of JU females.

METHOD

JU females, which had mated in post-partum oestrus, were allowed to suckle their first litters. Males were removed after post-partum mating. Females were subsequently assigned at random to one of three groups, which were treated as follows:

(i) Females were injected with 5 mg of progesterone ("Lutocyclin", CIBA Ltd.) on the 5th day post-coitum, and the dose was repeated every two days until the 17th day post coitum (designated "Treated").

(ii) Females were injected with oil at similar intervals (designated "Control").

(iii) Females were not injected (designated "Untreated").

The first litters were weaned and weighed at 21 days of age and females were sacrificed between 21 and 30 days post-coitum. The components of litter size were recorded at dissection, and the delay in implantation was calculated, as previously described.

RESULTS

Lactation was not suppressed by progesterone treatment, litters being weaned from all treated females except two, which consumed their live litters at 20 days. Progesterone treatment resulted firstly in successful pregnancies in all females treated (Table 25a). Secondly treatment resulted in a marked reduction in the duration of delay of implantation in concurrently lactating females. Implantation in most cases occurred one or two days after the first injection and only in one case was the delay greater than 3 days (Table 25a). Thirdly, treatment resulted in the effective increase of the size of second litters in lactating females, as estimated by the number of live young at term (Table 25b). As only one female in the oil-injected group was pregnant at dissection, this female was pooled with non-injected females to give some contemporary control data. Because of the paucity of control data, the increase of 2.6 live young on treatment was not significant. If two females of the treated group are excluded (because their uteri were contracted at autopsy) there is a significant difference in litter size between treated and control groups (difference = 3.5 ± 1.14, $p < 0.01$).

In terms of post-implantation mortality, the effect of progesterone was seen to reduce the proportion of later losses, especially in the "middle" period (Table 25a). Comparison of the frequencies of post-implantation losses in progesterone-treated females (Table 25a) with those of non-lactating females (Table 24) shows that treatment was effective. On dissection of progesterone-treated females, none of the characteristic symptoms associated with post-implantation death in lactating females, such as decidual necrosis, were noted.

DISCUSSION

The potential litter size of JU females in second parity, as judged by the number of corpora lutea, was greater than in first parity. The mean number of corpora lutea in first parity was found to be 11.0 $\pm$ 0.15 (McCarthy, 1963). In second parity, in the present studies, counts were nearer 12.0. It has been shown that the litter size of JU females was consistently lower in second parity and that this arose primarily as a result of the effect of concurrent suckling of first litter on the pre-natal survival of second litters. Many females did not become pregnant to post-partum mating. It was shown that concurrent lactation was responsible for whole-litter losses when first litters were removed at birth, this treatment resulting in a very high proportion of pregnancies. No direct evidence was found to determine whether whole-litter losses occurred because implantation did not take place or because all the conceptuses died in the post-implantation stage.

The effect of concurrent lactation was very marked in increasing post-implantation mortality in the period when the placenta was becoming established. Decidual necrosis was observed at dissection in most cases. Removal of first litters at birth demonstrated that concurrent lactation was responsible for this characteristic type of mortality. If progesterone was administered to lactating females, from the 5th day post-coitum, normal gestation of second litters ensued, implantation occurring shortly after the first injection, in all females which had mated in post-partum oestrus. The syndrome of "middle" post-implantation death was effectively absent in treated females. It would appear that the excess post-implantation deaths found in untreated lactating females was in no way attributable to

nutritional competition between the first litter , being suckled, and the second litter, being gestated.

One explanation which could be proposed is that delayed implantation, which was very marked in this stock, had a critical effect on the subsequent survival of the implanted embryos. Weichert and Schurgast (1942) found that, during delayed implantation in the rat, the growth of corpora lutea was postponed, but that subsequent implantation resulted in resumption of normal development of the corpora lutea. If delayed implantation, in JU females, did not result in normal resumption of corpus luteum development, then progesterone deficiency would occur. This could explain the heavy post-implantation mortality in lactating females. Henry and Venning (1938) proposed that progesterone was necessary for the maintenance of the decidua in a functional condition in humans. Lack of progesterone as suggested on the above hypothesis might explain the decidual necrosis observed in so many "middle" deaths after delayed implantation of second litter. On this hypothesis, heavier mortality might be expected with longer delays in implantation. When the delay in implantation was compared to the degree of mortality in litters, some slight relationship was seen (Table 26). Nevertheless, the frequency of post-implantation death in litters, which implanted after a delay of 7 to 8 days, was not appreciably less than that observed in litters where the delay was twice as long.

An attempt was made to test the above hypothesis. A small sample of JU females which had mated in post-partum oestrus, were injected with 7.5 mg. of progesterone on the fifth day post-coitum. It was thought that

this larger single dose would induce "undelayed" implantation. If the synchronisation of implantation and corpus luteum development was critical for optimal survival of the implants, further supplements of progesterone would be expected to be unnecessary. None of these females were pregnant at dissection. Progesterone treatment over part of gestation, at least, seems necessary for maximal implant survival in second litters of lactating females. A general antagonism between lactation and gestation, at a hormonal level, is probably responsible for the effects described in the JU strain. Without biochemical analysis, it is not possible to be more specific. The situation is atypical and is possibly due to inbreeding depression in some component of the physiological regulation of gestation in the JU stock.

SUMMARY

1. Concurrent lactation increased the frequency of whole-litter losses in JU females, which mated in the first post-partum oestrus.

2. Implantation of second litters was delayed by concurrent lactation.

3. Post-implantation mortality was increased in second litters, particularly in the "middle" period, by concurrent lactation.

4. Progesterone treatment shortened the delay in implantation, reduced the frequency of whole-litter losses and reduced the proportion of post-implantation mortality in second litters of concurrently lactating JU females.

5. An antagonism, at a hormonal level, between gestation and lactation is proposed as the basis of the phenomenon. This is probably a reflection of inbreeding depression in this stock.

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FIGURES AND TABLES

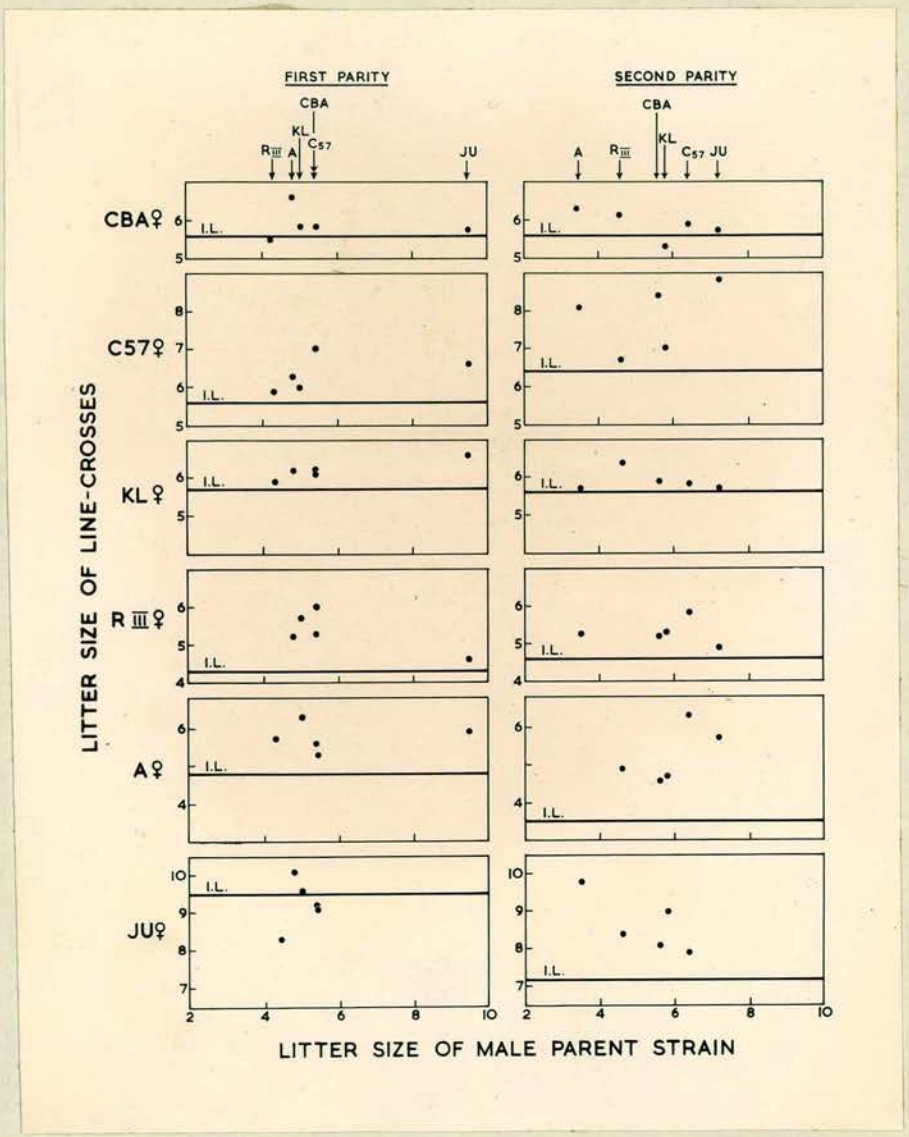

Fig. 1. The effect of crossing on litter size. The mean number of live young in crossbred litters is shown relative to the number of live young in inbred litters, in the same maternal environment (I.L. = Inbred Level).

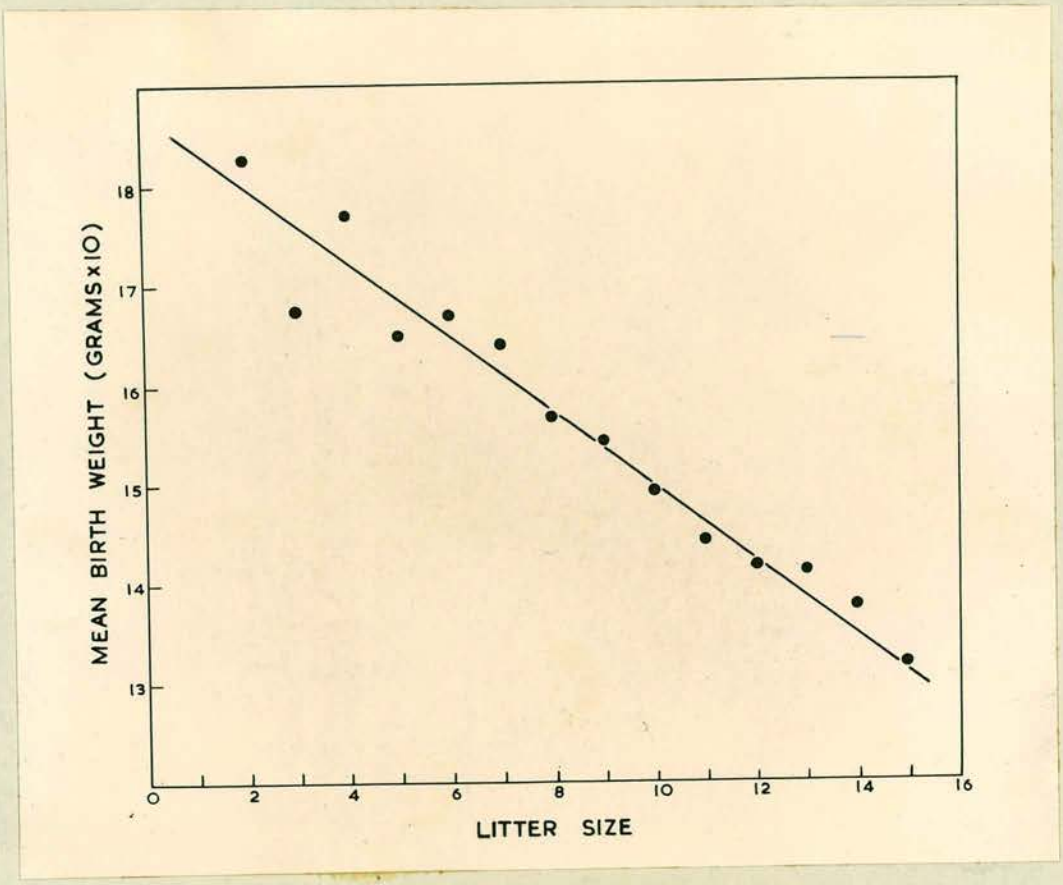

Fig. 2. The effect of litter size on birth weight. Mean birth weight of mice born in 440 litters of an outbred stock. The straight line is the computed weighted regression of birth weight on litter size at birth. The regression coefficient in b = -0.37 ± 0.03 (grams x 10).

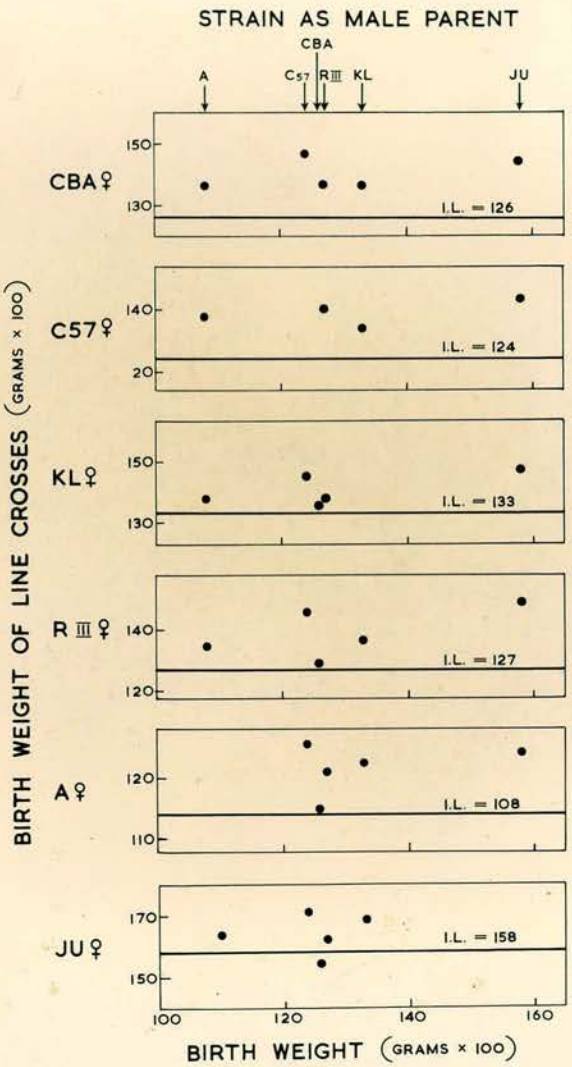

Fig. 3. The effect of crossing on birth weight. The mean birth weight of crossbred groups is shown relative to that of the inbred group in the same maternal environment (I.L. = Inbred Level).

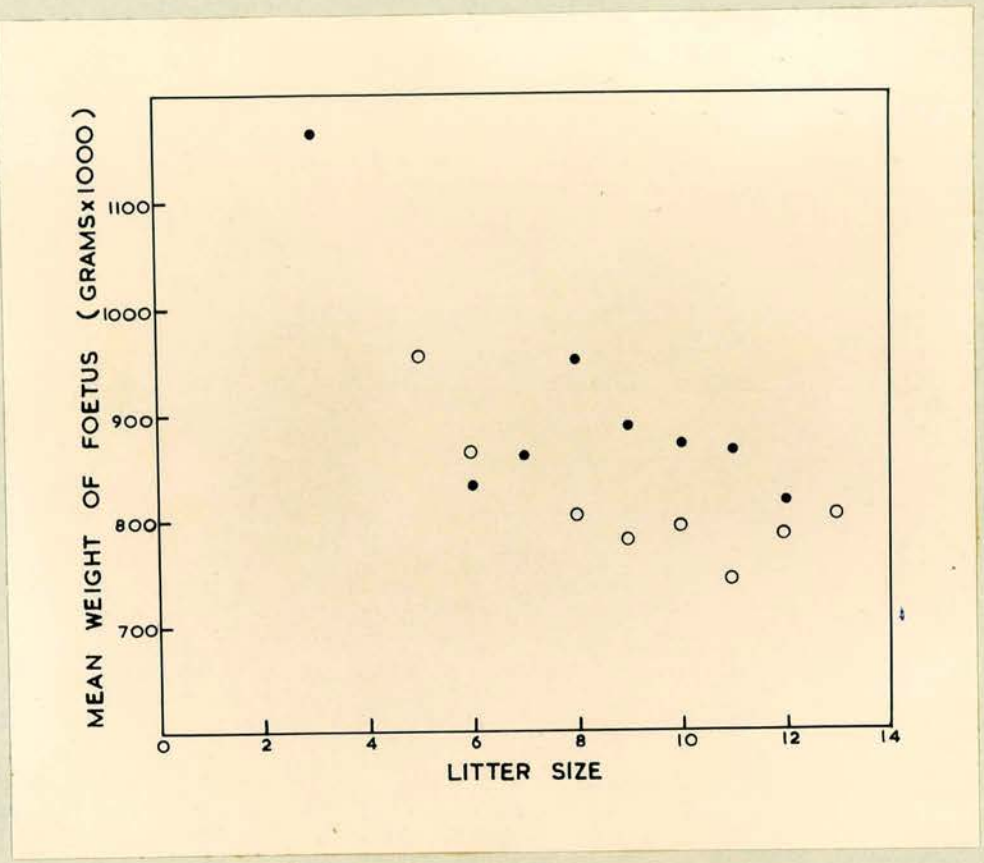

Fig. 4. Mean foetal weight plotted against the number of foetuses in the litter, for inbred litters (open circles) and crossbred litters (closed circles) of JU females. Each point is weighted by the number of inbred or crossbred litters at each litter size.

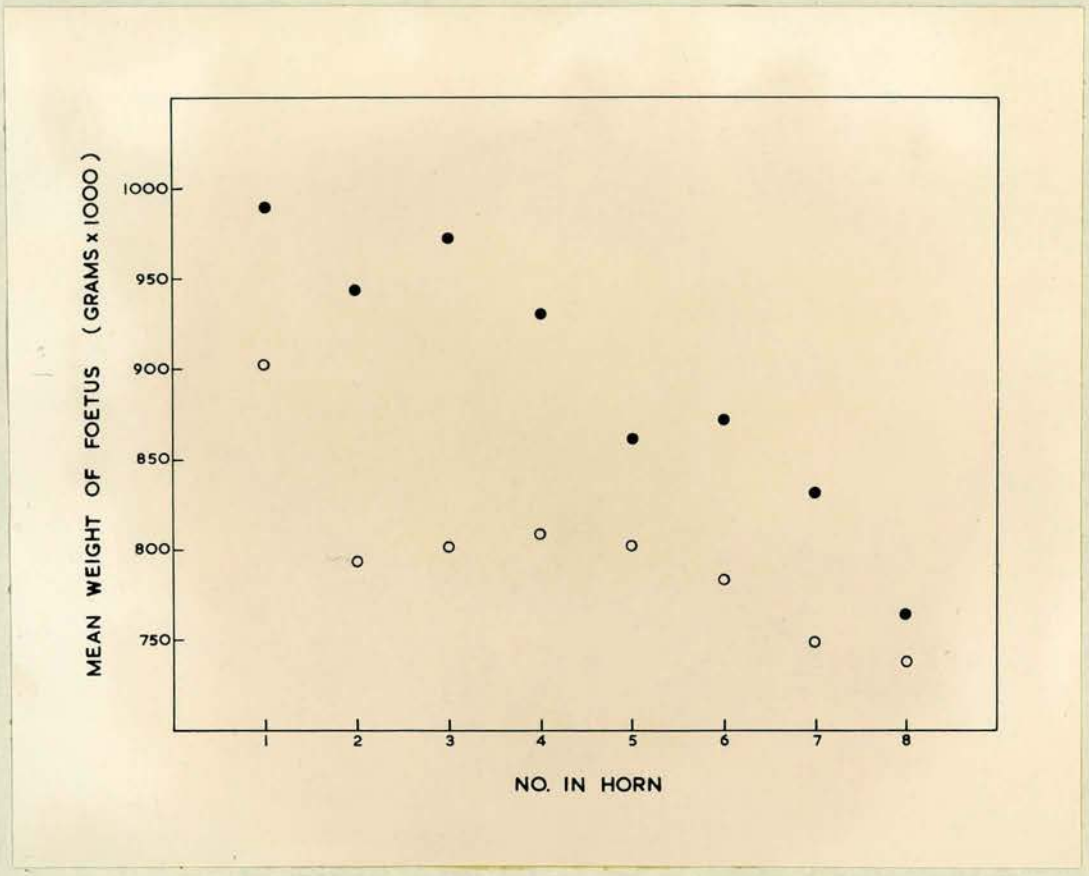

Fig. 5. Mean foetal weight plotted against the number of foetuses in the horn, for inbred litters (open circles) and crossbred litters (closed circles) of JU females. Each point is weighted by the number of inbred or crossbred litters in each number class.

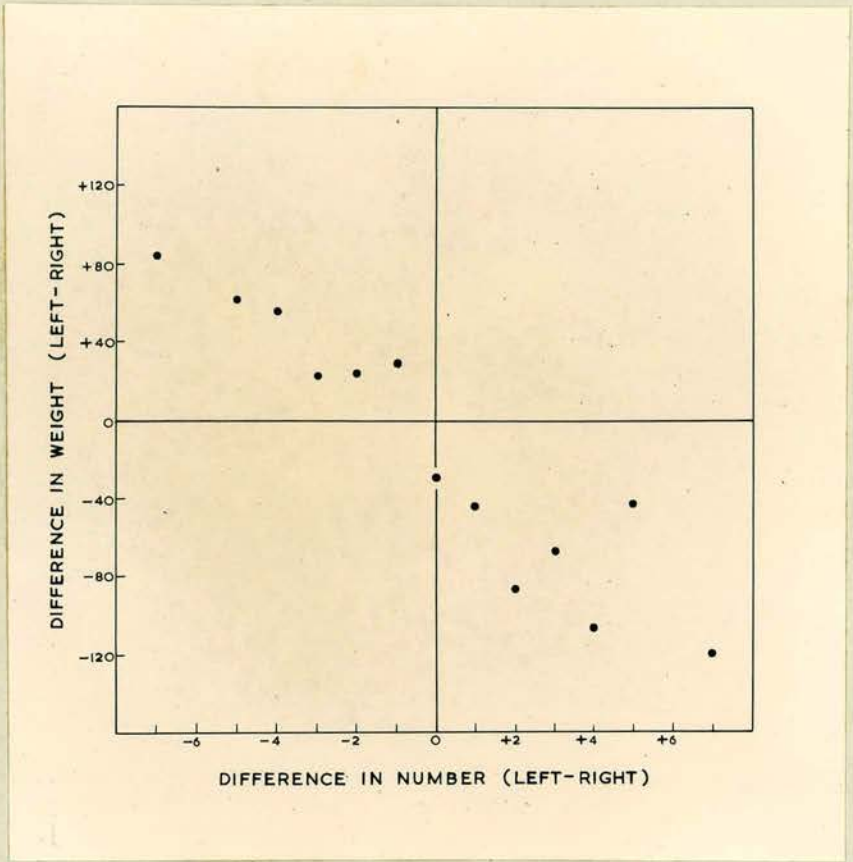

Fig. 6. The local effect of number in the horn on foetal weight in litters of JU females. The difference between the mean weight of foetuses in the left horn and in the right horn (grams x1000) is plotted against the difference between the number of foetuses in the left horn and in the right horn. Foetuses in the less crowded horn are heavier than those in the more crowded horn (shown in left half of graph) and vice versa.

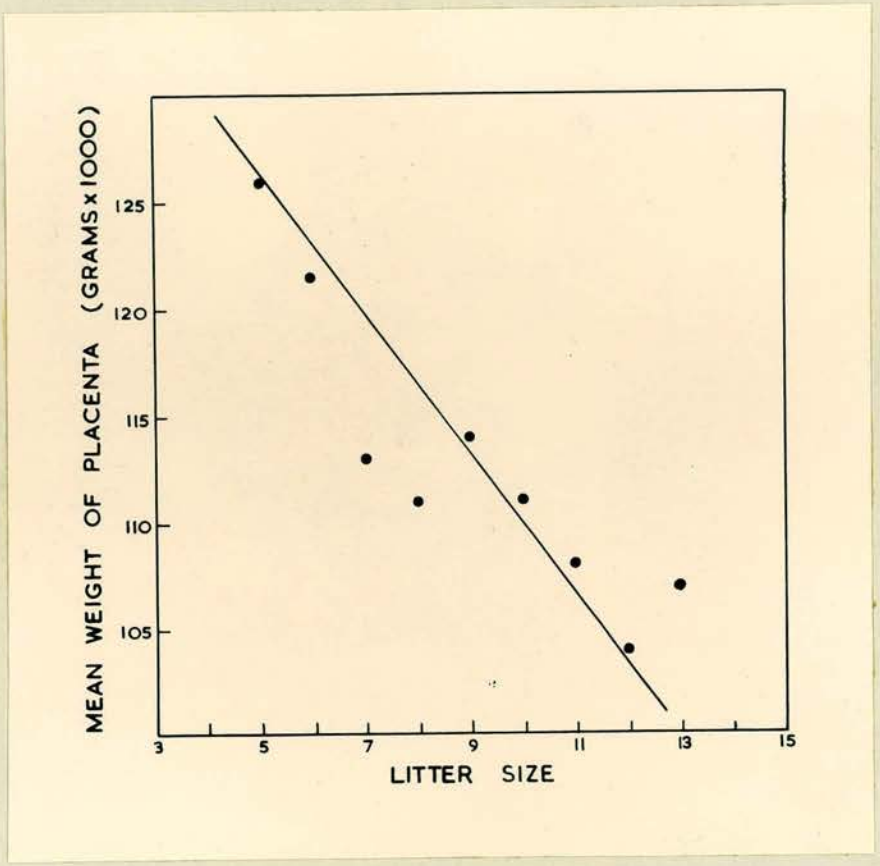

Fig. 7. Mean placental weight plotted against the number of feetuses in the litter for 107 litters of JU females. Each point is weighted by the number of litters at each litter size. The straight line is the computed weighted regression of placental weight on litter size. The regression coefficient is b = -2.4 ± 0.03 (grams x1000).

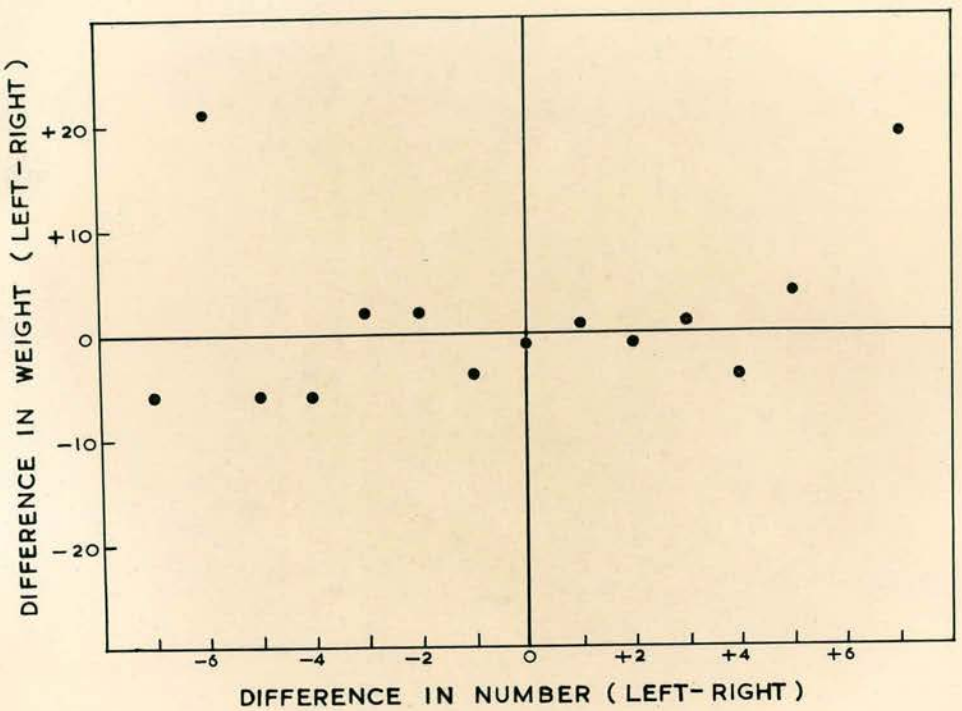

Fig. 8. The local effect of number in the horn on placental weight in litters of JU females. The difference between the mean weight of placentae in the left horn and in the right horn (grams x1000) is plotted against the difference between the number of foetuses in the left horn and in the right horn. Placentae in the less crowded horn are not heavier than those in the more crowded horn.

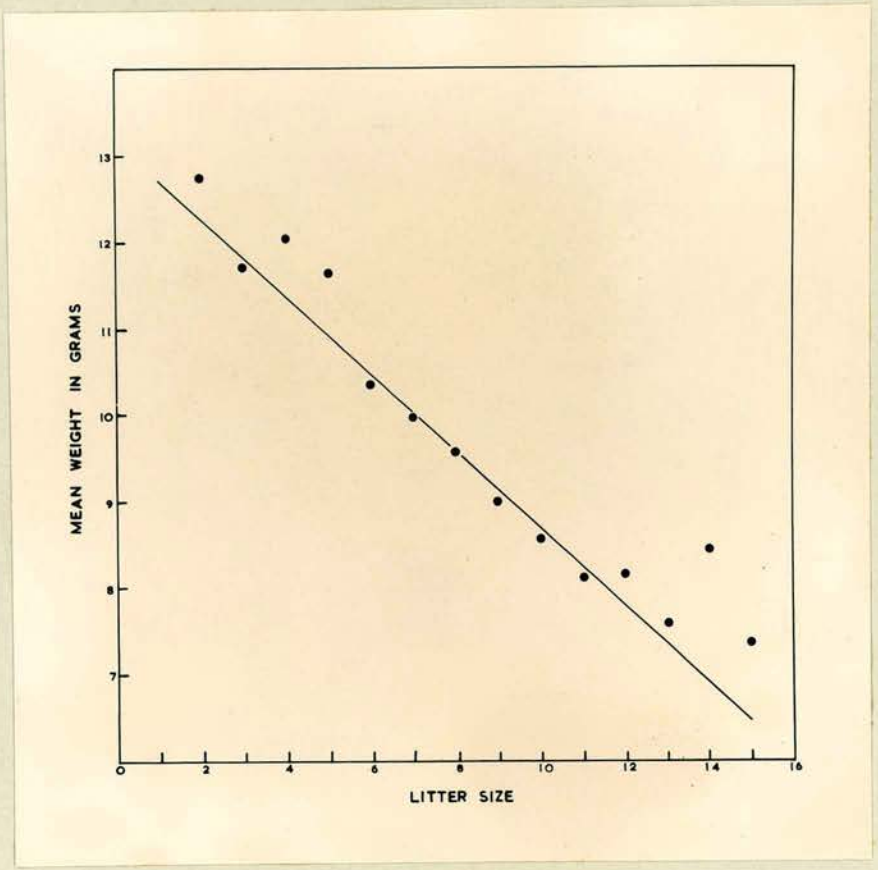

Fig. 9. The effect of litter size on weaning weight. Mean weight of mice weaned in litters of different sizes based on 1855 mice of an outbred stock. The straight line is the computed weighted regression of weaning weight on litter size at weaning. The regression coefficient is -0.45 ± 0.02 (grams).

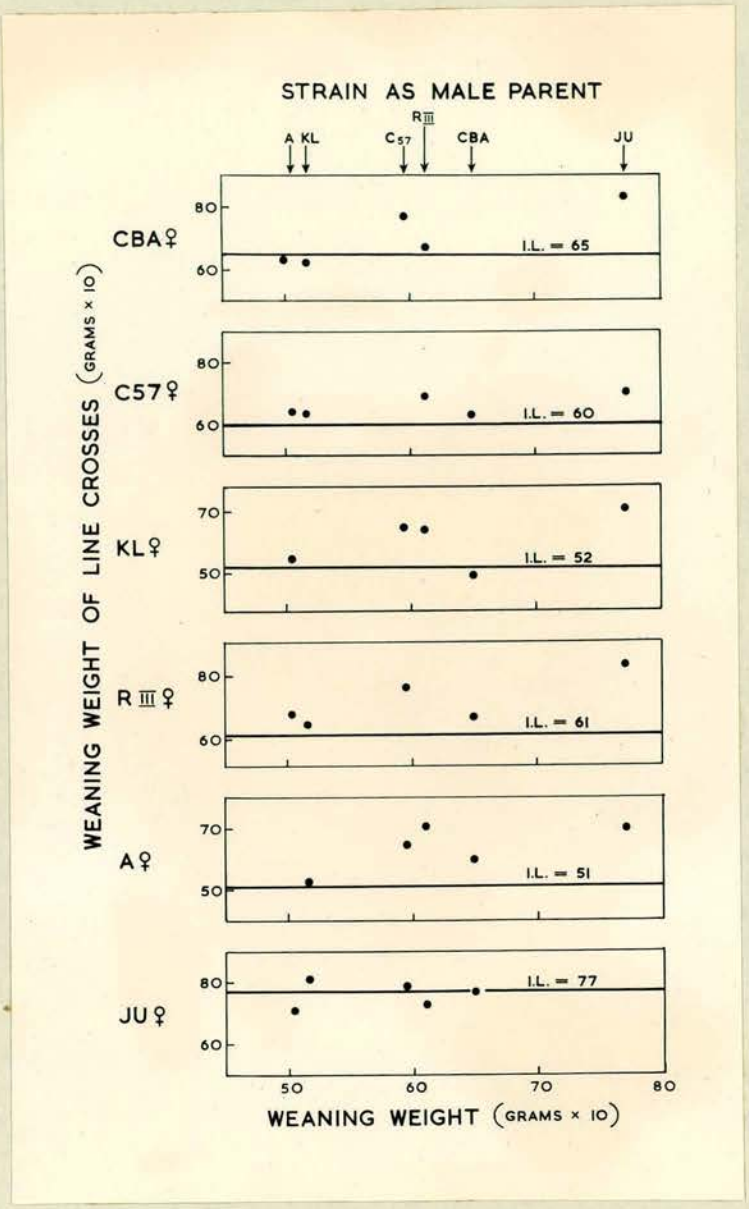

Fig. 10. The effect of crossing on weaning weight. The mean weaning weight of crossbred groups is shown relative to that of the inbred group in the same maternal environment (I.L. = Inbred Level).

TABLE 1

The total number of litters recorded in the six-by-six diallel crossing scheme

STRAIN AS FEMALE	STRAIN AS MALE: CBA	C57	KL	RIII	A	JU
CBA	65	40	38	57	55	42
C57	40	42	33	36	32	34
KL	43	37	58	41	38	40
RIII	41	35	35	45	38	44
A	24	25	14	27	34	25
JU	53	55	42	44	37	57

TABLE 2

Mean litter size of inbred strains and the crosses between them

FIRST PARITY.

STRAIN AS FEMALE ♀ ♂	CBA	C57	KL	RIII	A	JU
CBA	5.6 ± 0.3	5.8 ± 0.4	5.8 ± 0.3	5.5 ± 0.3	6.6 ± 0.2	5.7 ± 0.4
C57	7.0 ± 0.2	5.6 ± 0.3	6.0 ± 0.4	5.9 ± 0.4	6.3 ± 0.3	6.6 ± 0.3
KL	6.1 ± 0.4	6.2 ± 0.4	5.7 ± 0.4	5.9 ± 0.4	6.2 ± 0.4	6.6 ± 0.5
RIII	5.3 ± 0.3	6.0 ± 0.6	5.7 ± 0.5	4.3 ± 0.3	5.2 ± 0.3	4.6 ± 0.5
A	5.3 ± 0.5	5.6 ± 0.6	6.3 ± 0.3	5.7 ± 0.3	4.8 ± 0.3	5.9 ± 0.6
JU	9.1 ± 0.3	9.2 ± 0.5	9.6 ± 0.7	8.8 ± 0.3	10.1 ± 0.4	9.5 ± 0.3

SECOND PARITY.

STRAIN AS FEMALE ♀ ♂	CBA	C57	KL	RIII	A	JU
CBA	5.6 ± 0.3	5.9 ± 0.5	5.3 ± 0.4	6.1 ± 0.4	6.3 ± 0.3	5.7 ± 0.4
C57	8.4 ± 0.3	6.4 ± 0.4	7.0 ± 0.3	6.7 ± 0.5	8.1 ± 0.4	8.8 ± 0.4
KL	5.9 ± 0.5	5.8 ± 0.6	5.6 ± 0.5	6.4 ± 0.4	5.7 ± 0.4	5.7 ± 0.5
RIII	5.2 ± 0.5	5.8 ± 0.5	5.3 ± 0.6	4.6 ± 0.4	5.3 ± 0.5	4.9 ± 0.4
A	4.6 ± 0.5	6.3 ± 0.7	4.7 ± 0.8	4.9 ± 0.6	3.5 ± 0.4	5.7 ± 0.6
JU	8.1 ± 0.4	7.9 ± 0.5	9.0 ± 0.4	8.4 ± 0.4	9.8 ± 0.5	7.2 ± 0.5

TABLE 3

Mean litter size of all crossbred litters having a common strain as female parent compared with mean litter size of common parent strain.

Common strain as female parent	Parity	Litter Size Crossbred	Litter Size Inbred	Difference Crossbred-Inbred	Significance test
CBA	1	5.9 ± 0.15	5.6 ± 0.29	0.3 ± 0.32	N.S.
	2	5.9 ± 0.17	5.6 ± 0.30	0.3 ± 0.36	N.S.
C57	1	6.4 ± 0.15	5.6 ± 0.33	0.8 ± 0.35	$p < 0.05$
	2	7.7 ± 0.19	6.4 ± 0.39	1.3 ± 0.43	$p < 0.01$
KL	1	6.2 ± 0.18	5.7 ± 0.36	0.5 ± 0.40	N.S.
	2	5.9 ± 0.20	5.6 ± 0.53	0.3 ± 0.48	N.S.
RIII	1	5.3 ± 0.20	4.3 ± 0.34	1.0 ± 0.45	$p < 0.05$
	2	5.3 ± 0.22	4.6 ± 0.36	0.7 ± 0.49	N.S.
A	1	5.7 ± 0.22	4.8 ± 0.31	0.9 ± 0.40	$p < 0.05$
	2	5.2 ± 0.28	3.5 ± 0.39	1.7 ± 0.58	$p < 0.01$
JU	1	9.3 ± 0.20	9.5 ± 0.33	-0.2 ± 0.41	N.S.
	1*	9.1 ± 0.20	9.1 ± 0.17	0.0 ± 0.26	N.S.

* Supplementary series (see text).

TABLE 4

Mean numbers of corpora lutea, implants and live embryos at 17 days gestation (± standard errors). N is the number of females dissected per group.

Genotype of Females	Litters	N	Corpora lutea	Total implants	Live young	Difference in number of live young (crossbred-inbred)	
CBA	Inbred	53	7.7 ± 0.18	6.8 ± 0.18	5.8 ± 0.25	0.5 ± 0.34	N.S.
CBA	Crossbred	50	7.4 ± 0.14	6.9 ± 0.18	6.3 ± 0.22		
C57	Inbred	52	8.0 ± 0.18	7.1 ± 0.20	6.2 ± 0.25	0.3 ± 0.31	N.S.
C57	Crossbred	55	7.8 ± 0.14	7.1 ± 0.16	6.5 ± 0.18		
RIII	Inbred	58	7.4 ± 0.13	5.6 ± 0.25	4.3 ± 0.23	0.6 ± 0.35	> 0.05 P < 0.10
RIII	Crossbred	61	7.9 ± 0.14	5.6 ± 0.28	4.9 ± 0.26		
JU	Inbred	58	11.0 ± 0.15	10.0 ± 0.20	9.3 ± 0.21	-0.1 ± 0.30	N.S.
JU	Crossbred	54	10.8 ± 0.18	9.9 ± 0.23	9.2 ± 0.22		

TABLE 5

The incidence of post-implantation death to 17 days' gestation and the distribution of deaths between early (< 10 days) and later (10-17 days) stages in inbred and crossbred litters.

Genotype of Females	Litters	Total no.of implants	Total no.of deaths	% of implants		No.of early deaths	% of total implants		No.of later deaths	% of total implants
CBA	Inbred	361	55	15.2	)*	48	13.3	)*	7	1.9
CBA	Crossbred	344	30	8.7	)	27	7.8	)	3	0.9
C57	Inbred	370	46	12.4		43	11.6		3	0.8
C57	Crossbred	389	34	8.7		33	8.5		1	0.3
RIII	Inbred	327	79	24.2	)**	66	20.2	)***	13	4.0
RIII	Crossbred	345	44	12.8	)	31	9.0	)	13	3.8
JU	Inbred	584	47	8.1		38	6.5		9	1.5
JU	Crossbred	532	38	7.1		29	5.5		9	1.7

*** $P < 0.001$ that difference between ratios is due to chance.

** $P < 0.01$

* $P < 0.05$

TABLE 6

The effects of litter size and parity on mean birth weight in six strains (grams x100). The effects are estimated as the partial regressions of birth weight on litter size and litter order.

Strain as female parent	Effect of litter size (centigrams)	Effect of parity (Second - First) (centigrams)
CBA	-1.2 ± 0.53 *	+3.6 ± 1.8 *
C57	-2.7 ± 0.47 ***	+7.1 ± 1.7 ***
KL	-2.5 ± 0.38 ***	+2.8 ± 1.5 N.S.
RIII	-4.5 ± 0.50 ***	+7.0 ± 2.0 ***
A	-2.1 ± 0.68 **	+12.7 ± 2.6 ***
JU	-4.3 ± 0.30 ***	+4.9 ± 1.4 ***

*** $p < 0.001$

** $p < 0.01$

* $p < 0.05$

N.S. Not significant.

TABLE 7a

Mean birth weight of inbred and crossbred young adjusted to litter size 6 and first parity (grams x 100).

STRAIN AS FEMALE PARENT	STRAIN AS MALE PARENT: CBA	C57	KL	RIII	A	JU
CBA	126.4	146.6	135.9	135.8	136.5	144.7
C57	133.3	123.6	133.3	139.4	137.7	143.7
KL	135.2	144.1	132.5	136.4	137.1	146.5
RIII	128.8	145.1	136.1	126.5	134.4	148.4
A	110.4	132.0	125.6	122.8	108.4	128.1
JU	154.5	170.8	169.1	161.9	163.8	158.0

TABLE 7b

The differences between the mean birth weights of crossbred and inbred young having the same strain as female parent (grams x 100). Estimated by least squares as effects of genotype relative to the inbred level within common female parent. The mean weights of inbred young are given in the diagonal cells and are adjusted to litter size six and first parity.

		STRAIN AS MALE PARENT					
		CBA	C57	KL	RIII	A	JU
STRAIN AS FEMALE PARENT	CBA	126.4	*** +20.2	** +9.5	*** +9.4	*** +10.1	*** +18.3
	C57	*** +9.7	123.6	*** +9.6	*** +15.8	*** +14.1	*** +20.1
	KL	N.S. +2.7	*** +11.6	132.5	N.S. +3.9	N.S. +4.6	*** +14.0
	RIII	N.S. +2.3	*** +18.6	** +0.6	126.5	** +7.9	*** +22.0
	A	N.S. +2.0	*** +23.6	*** +17.2	*** +14.3	108.4	*** +19.7
	JU	N.S. -3.5	*** +12.8	*** +11.1	* +3.9	** +5.8	158.0

*** $p < 0.001$

** $p < 0.01$

* $p < 0.05$

TABLE 8

The mean weight of foetuses and of placentae at 17 days' gestation in inbred and crossbred litters of females of four inbred strains (grams x100). The mean weights are the means of the unweighted horn means. Standard errors are based on the variance of horn means.

Genotype of dam	Genotype of litter	No.of horns	Litter size	Mean foetal weight	Mean placental weight	Mean weight of dam (grams)
CBA	Inbred	96	5.8	71.1 ± 0.77	9.2 ± 0.10	16.1 ± 0.16
CBA	Crossbred (x C57 ♂)	89	6.3	81.0 ± 1.01 Diff. = 9.9 p < 0.001	10.6 ± 0.10 Diff. = 1.4 p < 0.001	16.1 ± 0.19
C57	Inbred	95	6.2	74.4 ± 0.87	9.6 ± 0.11	13.9 ± 0.19
C57	Crossbred (xCBA ♂)	104	6.5	84.9 ± 0.91 Diff.= 10.5 p < 0.001	11.1 ± 0.16 Diff. = 1.5 p < 0.001	13.8 ± 0.16
RIII	Inbred	106	4.5	69.8 ± 0.87	9.3 ± 0.14	15.6 ± 0.19
RIII	Crossbred (x JU ♂)	107	5.2	81.4 ± 0.95 Diff.= 11.6 p < 0.001	10.7 ± 0.13 Diff. = 1.4 p < 0.001	15.7 ± 0.21
JU	Inbred	110	9.3	79.5 ± 0.63	11.3 ± 0.09	16.3 ± 0.21
JU	Crossbred (x RIII ♂)	102	9.2	89.7 ± 0.86 Diff.= 10.2 p < 0.001	11.2 ± 0.12 Diff. = -0.1 N.S.	17.0 ± 0.18

TABLE 9

The increase in the mean weights of crossbred foetuses and placentae expressed as a percentage of the mean weights of inbred foetuses and placentae respectively.

Genotype of dam	Genotype of litter	Difference in Weight (Crossbred-Inbred)	
		Foetuses	Placentæ
CBA	CBA♀ x C57♂	14.0%	15.2%
C57	C57♀ x CBA♂	14.0%	15.6%
RIII	RIII♀ x JU♂	16.6%	15.1%
JU	JU♀ x RIII♂	12.8%	No increase

TABLE 10

Mean foetal weight and mean placental weight of crossbred young at 17 days' gestation in reciprocal crosses (grams x 100).

Cross No. ♀ ♂	Litter size	Mean foetal weight (centigrams)	Mean placental weight (centigrams)	Mean weight of dam (grams)
(1) CBA x C57	6.3	81.0 ± 1.01	10.6 ± 0.10	16.1 ± 0.19
(2) C57 x CBA	6.5	84.9 ± 0.01	11.1 ± 0.16	13.8 ± 0.16
Difference (2-1)		+3.9 ± 1.36	+0.5 ± 0.20	-2.3 ± 0.25
		$p < 0.01$	$p < 0.05$	$p < 0.001$
(3) RIII x JU	5.2	81.4 ± 0.95	10.7 ± 0.13	15.7 ± 0.21
(4) JU x RIII	9.2	89.7 ± 0.86	11.2 ± 0.12	17.0 ± 0.18
Difference (4-3)		+8.3 ± 1.28	+0.5 ± 0.18	+1.3 ± 0.29
		$p < 0.001$	$p < 0.01$	$p < 0.001$

TABLE 11

The local and systemic effects of litter size on the mean weight of foetuses in a horn shown by the partial regression coefficients b_1 and b_2, with weight of mother constant. The independent effect of weight of mother on foetal weight is shown by the partial regression coefficient b_3. (units = grams x100).

Series	Mothers	Litters	Number of horns	Effects on foetal weight (centigrams) Local (b_1)	Systemic (b_2)	Dam weight (b_3)
1	CBA	Inbred	96	-2.2 ± 0.65**	$+0.7 \pm 0.53$ N.S.	$+0.8 \pm 0.70$ N.S.
2	CBA	Crossbred	89	-1.7 ± 0.81*	-0.5 ± 0.76 N.S.	$+3.5 \pm 0.79$***
3	C57	Inbred	95	-0.5 ± 0.74 N.S.	-0.6 ± 0.65 N.S.	$+0.5 \pm 0.67$ N.S.
4	C57	Crossbred	104	-1.8 ± 0.67**	-0.5 ± 0.75 N.S.	$+1.4 \pm 0.78$ N.S.
5	RIII	Inbred	106	-1.5 ± 0.83N.S.	-1.1 ± 0.61 N.S.	$+0.7 \pm 0.54$ N.S.
6	RIII	Crossbred	107	-0.6 ± 0.79N.S.	-1.8 ± 0.57**	$+1.2 \pm 0.51$ *
7	JU	Inbred	110	-1.2 ± 0.39**	-1.5 ± 0.43***	$+1.0 \pm 0.38$**
8	JU	Crossbred	102	-2.1 ± 0.65**	-1.5 ± 0.59 *	$+1.2 \pm 0.60$ *

*** $p < 0.001$

** $p < 0.01$

* $p < 0.05$

N.S. Not significant

TABLE 12

The local and systemic effects of litter size on the mean weight of placentae in a horn shown by the partial regression coefficients b_1 and b_2, with weight of mother constant. The independent effect of weight of mother on placental weight is shown by the partial regression coefficient b_3. (units = grams x1000).

Series	Mothers	Litters	Number of horns	Effects on placental weight (milligrams) Local (b_1)	Systemic (b_2)	Dam weight (b_3)
1	CBA	Inbred	96	+0.2 ± 0.93 N.S.	-1.5 ± 0.75*	+1.7 ± 1.00 N.S.
2	CBA	Crossbred	89	+0.6 ± 0.92 N.S.	-0.9 ± 0.87 N.S.	+0.6 ± 0.90 N.S.
3	C57	Inbred	95	+1.6 ± 0.83 N.S.	-3.1 ± 0.74***	-0.8 ± 0.76 N.S.
4	C57	Crossbred	104	+0.8 ± 1.13 N.S.	-5.6 ± 1.26***	+2.7 ± 1.31 *
5	RIII	Inbred	106	+1.9 ± 1.27 N.S.	-4.9 ± 0.94***	+1.9 ± 0.83 *
6	RIII	Crossbred	107	+2.7 ± 1.03**	-4.3 ± 0.74***	+1.7 ± 0.67 *
7	JU	Inbred	110	+0.9 ± 0.61 N.S.	-2.0 ± 0.67**	-0.1 ± 0.59 N.S.
8	JU	Crossbred	102	+0.1 ± 0.89 N.S.	-3.8 ± 0.80***	+2.7 ± 0.83**

*** $p < 0.001$

** $p < 0.01$

* $p < 0.05$

N.S. Not significant.

TABLE 13

Supplementary series of JU litters. The effects of weight of the mother at insemination on foetal weight and placental weight, as measured by the partial regression coefficients of the mean weight of foetuses and of mean weight of placentae in the horn on weight at insemination. The effects of litter size are also shown.

Influence	Effects on foetal weight (grams x100)	Effects on placental weight (grams x1000)
Litter size		
Local (b_1)	-1.1 ± 0.45 *	$+1.3 \pm 0.80$ N.S.
Systemic (b_2)	-1.6 ± 0.48 ***	-2.9 ± 0.85 **
Weight of dam (b_3) at insemination.	$+1.3 \pm 0.50$ **	$+0.9 \pm 0.90$ N.S.

*** $p < 0.001$

** $p < 0.01$

* $p < 0.05$

N.S. Not significant.

TABLE 14

The local and systemic effects of litter size (b_1 and b_2), the effect of weight of mother (b_3) and the effect of placental weight (b_4) on foetal weight (grams x100).

Series	Effects on foetal weight (centigrams)			
	Local (b_1)	Systemic (b_2)	Dam weight (b_3)	Placental weight (b_4)
1	-2.2 ± 0.65**	+0.8 ± 0.53 N.S.	+0.8 ± 0.73 N.S.	+0.9 ± 0.73 N.S.
2	-1.9 ± 0.79*	-0.3 ± 0.75 N.S.	+3.5 ± 0.78***	+2.1 ± 0.93*
3	-0.6 ± 0.75 N.S.	-0.4 ± 0.71 N.S.	+0.6 ± 0.68 N.S.	+0.7 ± 0.93 N.S.
4	-1.9 ± 0.67**	-0.2 ± 0.90 N.S.	+1.2 ± 0.80 N.S.	+0.5 ± 0.59 N.S.
5	-1.9 ± 0.81*	-0.2 ± 0.67 N.S.	+0.4 ± 0.54 N.S.	+1.8 ± 0.62**
6	-1.1 ± 0.79 N.S.	-0.9 ± 0.63 N.S.	+0.9 ± 0.51 N.S.	+2.1 ± 0.73**
7	-1.2 ± 0.40**	-1.4 ± 0.45**	+1.1 ± 0.38**	+0.7 ± 0.63N.S.
8	-2.3 ± 0.72**	-0.7 ± 0.63 N.S.	+0.7 ± 0.62 N.S.	+1.9 ± 0.72**

*** $p < 0.001$

** $p < 0.01$

* $p < 0.05$

N.S. Not significant.

TABLE 15.

The mean foetal weights and mean placental weights of first and second litters of JU females (grams x100). Series (a) and (b) in second parity refer to dissections made 17 days after post-partum and post weaning plugs, respectively.

Parity	No.of horns	Litter size	Mean foetal weight weight (centigrams)	Mean placental weight
First	110	9.3	79.5 ± 0.63	11.3 ± 0.09
Second (a)	50	9.4	78.0 ± 1.90	12.8 ± 0.16
(b)	68	8.8	74.2 ± 0.83	13.3 ± 0.17
Differences in weight:				
First - Second (a)			+1.5 ± 1.55 N.S.	-1.5 ± 0.17***
First - Second (b)			+5.3 ± 1.02***	-2.0 ± 0.17***
Second (a) - Second (b)			+3.8 ± 1.83*	-0.5 ± 0.23*

*** $p < 0.001$

* $p < 0.05$

N.S. Not significant

TABLE 16a

The mean weaning weight of inbred and crossbred young, adjusted to litter size 6 and first parity (grams).

STRAIN AS FEMALE PARENT	STRAIN AS MALE PARENT: CBA	C57	KL	RIII	A	JU
CBA	6.49	7.64	6.20	6.72	6.34	8.35
C57	6.16	5.97	6.37	6.87	6.41	7.00
KL	4.95	6.50	5.19	6.38	5.46	7.05
RIII	6.65	7.61	6.40	6.12	6.72	8.27
A	5.95	6.52	5.08	7.03	5.05	6.96
JU	7.61	7.87	8.05	7.68	7.13	7.73

TABLE 16b

The differences between the mean weaning weight of crossbred and of inbred young, having the same strain as female parent (grams). The mean weights of inbred young are given in the diagonal cells and are adjusted to litter size six and first parity.

		STRAIN AS MALE PARENT					
		CBA	C57	KL	RIII	A	JU
STRAIN AS FEMALE PARENT	CBA	6.49	*** +1.15	N.S. -0.29	N.S. +0.23	N.S. -0.15	*** +1.86
	C57	N.S. +0.19	5.97	N.S. +0.40	** +0.90	N.S. +0.44	*** +1.03
	KL	N.S. -0.24	** +1.31	5.19	* +1.19	N.S. +0.27	*** +1.86
	RIII	N.S. +0.53	*** +1.49	N.S. +0.28	6.12	N.S. +0.60	*** +2.15
	A	N.S. +0.90	* +1.47	N.S. +0.03	** +1.98	5.05	** +1.91
	JU	N.S. -0.12	N.S. +0.14	N.S. +0.32	N.S. -0.05	N.S. -0.60	7.73

*** $p < 0.001$

** $p < 0.01$

* $p < 0.05$

N.S. Not significant.

TABLE 17

Ingredients of stock diet. Vitamins in lower third of table were included in 1960.

Millers Offals	20.0 %
Ground Oats	17.5 %
Maize meal	18.8 %
Barley meal	8.8 %
Meat and Bone meal	8.8 %
Fish meal	5.0 %
Dried Skimmed Milk	7.5 %
Whey Powder	6.3 %
Unextracted Dried Yeast	1.3 %
Molasses	5.0 %
	per ton
Salt (NaCl)	9 lbs.
Vit. A (in gelatin base)	12 million units
Vit. D	3 million units
ADDED IN 1960	
Riboflavin	2.5 grammes
D. calcium pantothenate	5 grammes
Aneurin	0.85 grammes
Niacin	12.5 grammes
Pyridoxin	0.25 grammes
Choline Chloride	100 grammes
Vitamin 'B 12'	1,500 microgrammes
Vitamin 'E'	2.54 grammes
Vitamin 'K' (Menadione bi-sulphate)	1.5 grammes

TABLE 18

The mean weaning weight of crossbred and inbred litters of JU females in three different post-natal environments. N is the mean number of young weaned in litters of each group.

Group	Post-natal environment	Crossbred		Inbred		Difference in weight (Crossbred - Inbred)
		N	Mean weight	N	Mean weight	
1. Fostered	Outbred ♀♀ (standard diet)	9.0	9.5 ± 0.36	9.0	8.3 ± 0.26	+1.2 ± 0.44**
2. Control	JU ♀♀ (standard diet)	8.4	6.8 ± 0.21	8.0	6.4 ± 0.17	+0.4 ± 0.28 N.S.
3. Minimal	JU ♀♀ (Oats and maize)	7.8	3.9 ± 0.19	7.2	4.0 ± 0.18	-0.1 ± 0.26 N.S.

** $p < 0.01$

N.S. Not significant.

TABLE 19

A comparison between the size of first and second litters of inbred females of JU stock (1960-1962)

Class	Parity	No. of litters	Mean litter size	Difference First minus Second
Diet A	First	42	8.0 ± 0.3	1.3 ± 0.5*
	Second	42	6.7 ± 0.4	
Diet B	First	42	9.1 ± 0.3	1.9 ± 0.5**
	Second	42	7.2 ± 0.4	
Stock 1	First	25	9.9 ± 0.4	2.1 ± 0.7**
	Second	25	7.8 ± 0.5	
Stock 2	First	23	9.0 ± 0.3	1.8 ± 0.8*
	Second	23	7.2 ± 0.5	

* $p < 0.05$ ** $p < 0.01$.

TABLE 20

Analysis of variance of the size of second litters in four groups of JU stock.

Source of variation	df	S.S.	M.S.	F.
Between groups	3	21.73	7.24	1.06 N.S.
Within groups	128	877.82	6.86	
Total	131	899.55		

TABLE 21

The numbers of pregnant lactating and non-lactating females (uncomplicated) dissected, the mean age of live embryos and the delay in implantation in the lactating group.

Conditions of pregnancy	No. of females	Mean age of live embryos.	Delay in implantation Mean	Range
Concurrent lactation	43	14.1 days	9.8 days	5-17 days
Uncomplicated	36	All 17 days	-	-

TABLE 22

The distribution of the duration of the delay in implantation and the mean number of young suckled by 43 females, in which gestation and lactation were concurrent.

No. of litters	4	4	8	3	1	4	3	10	4	2
Delay in days	5	6	7	8	9	10	11	12 and 13	14 and 15	16 and 17
Mean no. weaned	6.3	7.0	7.4	8.7	4.0	7.3	9.0	8.5	8.5	9.5

TABLE 23

The mean number of corpora lutea, live young, the pre-implantation mortality, as a percentage of corpora lutea, and the incidence of post-implantation mortality, as a percentage of the total number of implantations, in second pregnancies of lactating and non-lactating females (uncomplicated).

Conditions of pregnancy	No.of pregnant females	Mean no. of corpora lutea	Mean no. of live young	Pre-implant loss % corp.lutea	Post-implant loss % total implants
Concurrent lactation	43	11.8 ± 0.20	5.4 ± 0.38	9.2	48.3
Uncomplicated non-lactating	36	11.7 ± 0.24	8.6 ± 0.37	16.2	11.6
First litters removed non-lactating	28	12.4 ± 0.25	9.1 ± 0.41	11.8	17.0

TABLE 24

The incidence of post-implantation death and the distribution of deaths between "early", "middle" and "late" stages, in lactating and non-lactating females.

Conditions of pregnancy	Total no. of deaths	% of total implants	No.of early deaths	% of total implants	No. of middle deaths	% of total implants	No.of late deaths	% of total implants
Concurrent lactation	217	48.3%	86	19.2%	117	26.0%	14	3.1%
Uncomplicated non-lactating	41	11.6%	30	8.5%	7	2.0%	4	1.1%
First litters removed non-lactating	52	17.0%	42	13.7%	7	2.3%	3	1.0%

TABLE 25a

The effects of progesterone treatment on:
(1) the proportion of females becoming pregnant to post-partum insemination;
(2) the duration of delay in implantation, and
(3) the proportion of post-implantation losses, in second litters of concurrently lactating JU females.

Group	No. in group	Pregnant No.	Pregnant %	Delay in implantation	Post-implantation mortality Total %	Early %	Middle %	Late %
Treated	19	19	100	1 to 5 days	21.6	18.2	2.4	1.0
Control	19	1	5	8 to 14 days*	45.0	7.0	29.0	9.0
Untreated	14	5	36					

* pooled estimates for controls.

TABLE 25b

The number of live young at dissection in progesterone-treated and control lactating JU females.

Group	No.of pregnancies	Live young	No. of pregnancies	Live young
Treated	19	8.6 ± 0.71	17	9.5 ± 0.11
Control (pooled)	6	6.0 ± 1.20	6	6.0 ± 1.20

N.S. (between 8.6 ± 0.71 and 6.0 ± 1.20)

* (between 9.5 ± 0.11 and 6.0 ± 1.20)

* Difference 3.5 ± 1.14 $p < 0.01$.

TABLE 26

The relationship between the duration of delay in implantation and the degree of post-implantation mortality in untreated lactating JU females. ("Total" mortality is the percentage of all implants, in each class, represented by dead implants at dissection).

No.of litters	8	13	7	8	10	-	2
Delay in days	5-6	7-8	9-10	11-12	13-14	15-16	17-18
"Total" mortality	36%	45%	47%	45%	60%	-	55%